The AVITAMINOSES

The Chemical, Clinical and Pathological Aspects of the Vitamin Deficiency Diseases

By

WALTER H. EDDY, PH.D.

Emeritus Professor of Physiological Chemistry, Teachers College, Columbia University;

AND

GILBERT DALLDORF, M.D.

Pathologist of the Grasslands and Northern Westchester Hospitals, Westchester County, New York

THIRD EDITION

"For it is now clear to anyone who will study the evidence, that nutrition has greater constructive potentiality than science had foreseen, and that even in the everyday choice of food we are dealing with values which are above price, for the health and efficiency, duration and dignity, of human life."

—HENRY C. SHERMAN

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PREFACE TO THE THIRD EDITION

Continued reader interest has justified a third edition. While there have been no changes in our purpose the text has been rearranged to sharpen the separation between the deficiency diseases and the chemical nature and functions of the vitamins. We have thus been able to eliminate some of the repetition present in the previous editions.

We hope very much that the discussion of the significance of vitamins as disease provokers (Chapter XV) does not convey an impression of omniscience. We subscribe to the sound point of view of the technical committees of the American Medical Association which we believe reflects the judgment of many clinicians. Inevitably their opinions do not attract the attention that more enthusiastic views do. One might say that as yet there is no reason to assign the many known physical, chemical and living causes of disease to a secondary role. We would not be surprised if that did happen for we know the limitations of our own judgment. Many more facts and much less opinion should help.

The chapters on the chemical nature of the vitamins and cellular oxidation have been rewritten and much new material has been added including many new illustrations.

We acknowledge with thanks the assistance of Professor W. C. MacTavish.

G. D.
W. H. E.

PREFACE TO FIRST EDITION

This book is directly derived from "The Vitamine Manual" which the senior author wrote fifteen years ago. But the scope of the book has been enlarged by the addition of both pathological and clinical discussions of the vitamin deficiency diseases. How this came about may be of interest for it mirrors the transitional development of the subject. Eight years ago the senior author brought to the pathological laboratory of the old New York Hospital some guinea pigs he suspected of being scorbutic although there was nothing in their gross appearance to support such a diagnosis. In this way did the advantages of joint pathological and biochemical studies become apparent to us and the association begin which has led us to write this book.

The problems in which we have since collaborated have dealt particularly with the development of anatomical criteria by which to evaluate the results of dietary experiments and, more recently, the application of this knowledge to the practice of medicine including the search for anatomical evidence of deficiency disease in human tissues at autopsy and in the wards and clinics.

The book has been planned to be a helpful manual rather than a complete treatise. In addition to the current views concerning the nature and functions of the vitamins we have tried to assemble in one volume what is known of the pathological anatomy and clinical aspects of the diseases due to their insufficiency, to describe clinical methods we have found useful in studying human cases and to present tables of vitamin values of all those foods which have been assayed expressed not in plus signs but in units per ounce so that diets can be more precisely measured for their vitamin value.

While we have not hesitated to interject our own views of controversial subjects we believe we have identified them as being our own clearly enough that the reader may subtract them from the rest of the book and still retain a resume of current opinion of other workers.

Our hope is that the book may be as useful to others as the many workers in this field have been to us in helpful advice and other generous services. The most pleasurable experience which has come to us in the writing of the following pages has been due to the universal friendliness and helpful interest of our colleagues. It is a rich and undeserved reward. We thank them all at this time.

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FOREWORD

The preparation of the first comprehensive treatise in English on the pathological responses to vitamin deficiencies emphasizes the importance of the application of morphological control in the study of vitamins. It emphasizes the commanding position of morphology in the interpretation of disease processes. The occurrence of a great variety of degenerative diseases of organs and mucous membranes, of peculiar character and obscure etiology, was long recognized as one of the mysteries of pathology. Rickets, scurvy, beriberi and pellagra were but the more prominent of these diseases. Their general features were fully recognized but their causation remained undetermined until the introduction of the doctrine of vitamins. Then began, on a vast scale, the experimental study of vitamins, yielding a volume of new facts of great scope, but lacking coordination and uniformity. This situation was mainly due to the lack of any delicate and reliable indicator for vitamin deficiency.

Commencing with the work of Wolbach and Howe in 1925–1928, demonstrating the specific changes in the morphology of various epithelial tissues due to vitamin A deficiency, the value of pathological examination as a delicate indicator of the presence or absence of adequate vitamins was demonstrated and extensive studies were stimulated. It is in this field that the authors of the present treatise have made important contributions and the present volume demonstrates how these methods have served to bring order out of chaos.

The enormous literature on vitamins has been collated and digested and reliable conclusions presented in condensed and accessible form for the general physician and pathologist. It will now be possible to determine more accurately how frequently deficiency diseases occur among the sick in general and hospital practice. A reliable clue is also available to the general pathologist in the interpretation of various morbid conditions, metaplasia, disorders of collagen formation, proliferative changes of non-inflammatory type, degenerative changes in the nervous system, etc.

The book is comprehensive in its inclusion of a discussion of the nature of the various vitamins and their functions as well as other information of a very practical nature. The appearance of such a work, therefore, is to be cordially welcomed, and the authors congratulated on its preparation.

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PART TWO. THE AVITAMINOSES

PART THREE. TECHNICAL METHODS, VITAMIN ASSAY AND VITAMIN VALUES

PART ONE

THE VITAMINS

PART ONE

THE VITAMINS

identified only by animal behavior and still await isolation and confirmati of claims. Probably others exist that are not yet even postulated.

On page 4, Table I, is a list of vitamins known today to actually ex and also others simply postulated and awaiting proof of existence. page 5 (see Table II) is also a tabulation of the units of measureme that have been used to express vitamin potency and quantity.

TABLE III

Terms Used to Express Vitamin Potency and Their Equivalence

Weight of vitamins is today generally given in milligrams or micrograms (1/100 of a milligram). To understand these values note:

One ounce Avoirdupois = 28 grams, 28,000 milligrams or 28,000,000 micrograms

One microgram = 1 gamma.

VITAMIN A

1 Int. or U.S.P. Unit = 0.0006 mgm. beta-carotene
1 Int. or U.S.P. Unit = 0.7 Sherman-Munsell unit

VITAMIN B_1

1 Int. or U.S.P. Unit = 0.003 mgm. thiamine
1 Int. or U.S.P. Unit = 2 Sherman Chase units
1 Int. or U.S.P. Unit = 0.5 Smith Curative units
1 Int. or U.S.P. Unit = 1.0 Chick-Roscoe units
1 Int. or U.S.P. Unit = 20 Cowgill mgm.-equivalents

VITAMIN C

1 Int. or U.S.P. Unit = 0.05 mgm. l-ascorbic acid
1 Int. or U.S.P. Unit = 0.1 Sherman-LaMer unit

VITAMIN D

1 Int. or U.S.P. Unit = 0.000025 mgm. calciferol
1 Int. or U.S.P. Unit = 1.37 Steenbock units
1 Int. or U.S.P. Unit = 1 A D M.A. unit
1 Int. or U.S.P. Unit = 1 Oslo or Poulsson unit

ORIGIN OF VITAMINS

Funk compounded the word "vitamine" of the prefix "vita" and th suffix "amine" because his original crystals contained basic or amin nitrogen and were essential to life. Moreover, "it was necessary for me t choose a name that would sound well and serve as a catchword." Th term was modified by dropping the final "e" at the suggestion of Drummon when it was learned that an amine group was not a characteristic of a "vitamines." The common practice of designating the various vitamin by letters is a sequel of the pioneer nutritional studies which started at th University of Wisconsin in 1907 as an investigation of the value of cereal in animal nutrition and which were later transferred to Johns Hopkin

University. There, in 1913, McCollum and Davis demonstrated a growth factor in the nonsaponifiable fraction of the fat from butter and egg yolk which they called "unidentified dietary factor, fat-soluble A."

At present three methods are used in designating the vitamins. Thus the substance which Funk originally called "vitamine" is known as water soluble vitamin B_1, the antiberiberi or anti-neuritic vitamin or as thiamine in this country and aneurin on the continent. The latter terms are the chemical names for the substance and it is probable that as the various vitamins are identified chemically their chemical names will replace the terms by which we first knew them.

As shown in the following chapter, there is little structural resemblance between the individual vitamins, vitamin A having quite a different chemical configuration than that of vitamin B_1 or vitamin D. Vitamin is a functional designation not a chemically descriptive term.

Schopfer defines a vitamin as follows: "An organic substance, the need for which results from the loss of capacity for synthesis, whose action is catalytic (active in small amounts), quantitative, and markedly specific."

It is convenient to consider them as a functional group for they share certain features. Thus they are present, and active, in minute amounts, in contrast to the considerable quantities of the ordinary nutrients. They also differ from the latter in that some may be inactivated by heat or oxidation. They have been discovered and studied by a common technique, that of devising basal diets of purified constituents deficient only in the factors under investigation and so measuring the effects of deprivation and of supplements. Because they resemble in their behavior the products of the glands of internal secretion, such as thyroxin and insulin, and are present in foods they are sometimes called food hormones. Of recent years the importance of certain vitamins to plant growth and development has suggested that they may represent the equivalent, in the vegetable kingdom, of the hormones of the animal kingdom and thus be both plant hormones as well as food hormones.

A further characteristic of certain vitamins is that they may occur in natural sources in a physiologically inactive form (provitamins) which become active only after conversion within the animal or by other means. Thus vitamin A exists in plants as a yellow pigment, carotene, which is activated in the liver. The D vitamins are the result of ultra violet light activation of parent substances; calciferol, for example, by ultra violet irradiation of ergosterol.

The avitaminoses are as ill assorted a group of diseases as the vitamins are ill assorted chemically. But they, too, share enough common characteristics to justify their inclusion in a single group of diseases. They are due to the *absence* of minute amounts of biologically important materials

rather than to the *presence* of minute amounts of infectious agents. Vitamin deficiency does not cause disease in a positive but in a *negative sense.* Pathogenic organisms produce chemical and morphological damage; vitamin deficiency removes an essential ingredient from the physiological equation. The deficiency *is* the disease. It is true that many deficiency diseases are recognized by the compensatory mechanisms of the body. Thus in riboflavin deficiency the cornea becomes vascularized because, it is thought, the natural method of oxygen exchange in that part is suppressed. But these are secondary manifestations of the disease.

A third characteristic of the deficiency diseases is that they may be present in any degree. One may suffer a latent infection but not be partially infected. A malignant growth is present or not. But the deficiency diseases may occur in such partial forms as to raise a question as to whether disease is actually present. This is, indeed, the most pressing problem in the field, for all evaluation of what constitutes a proper diet rests upon it. The avitaminoses are also commonly present in mild degree; a feature which more than any other delayed recognition of their importance. In these incipient forms the lesions and symptoms are difficult to recognize; the effects of treatment less obvious and uncertainties bound to remain. *They exist as a disturbance of the substrate, as a coloration* of the physiological and structural constitution which, while of great importance in balancing the scales of health and in modifying the responses to noxious agents, remains hidden from view.

A further characteristic is that they are seldom the immediate cause of death. One searches the mortality records in vain for clues to their importance and in the dissecting room they receive scant attention. They are contributory causes of death but seldom the immediate cause. Despite the little attention they receive in necropsy protocols those deficiency diseases which are well understood from a morphological point of view have characteristics as unique and specific as the structure of the vitamins themselves.

It has become obvious that the deficiency diseases are a much more complex problem for the physician than for the biochemist since the manifestations of deficiency may be influenced by other diseases just as other diseases modify vitamin requirements, by altering the efficiency of assimilation and suppressing or emphasizing the importance of dietary deficiencies. There is, indeed, a special field of nutrition which is strictly limited to the *practice of medicine.*

Chapter II

CHEMICAL NATURE OF THE VITAMINS

The chemical nature of the vitamins that have been identified to date is described in the following pages. In general, it is of interest to note that they are quite unrelated chemically; that the term "vitamin" indicates performance rather than similarity of chemical structure.

THE CHEMICAL NATURE OF VITAMIN A

Clue to the nature of this vitamin came with the demonstration that it occurs in plant sources as a yellow pigment; first identified by Wachenroder in 1826 and called by him, "carotene," because he obtained the pigment from carrots. Steenbock in this country was the first to suggest that there might be a relation between carotene and vitamin A. But proof that carotene is provitamin A is due primarily to the work of Moore and of Karrer and associates.

There are a number of these carotenoid pigments present in plants. Some of them are not convertible into vitamin A but at least four can be so changed in the animal body. These four are alpha, beta and gamma-carotene and cryptoxanthin and of these four, the beta-carotene is most potent, originally believed to yield on conversion, 2 molecules of vitamin A while the other three were said to produce only 1 molecule each. That view has changed, as explained in the following pages. The active vitamin A exists in two forms which are today designated as A_1 and A_2. A_1 is the form found in the liver oils of salt water fish; A_2 in the liver oils of fresh water fish.

The term "vitamer" was first suggested to designate vitamins with similar function. R. J. Williams has contended that a better word is "isotel." A_1 and A_2 then are vitamers or isotels, whichever designation is adopted.

To Karrer and his associates we owe the determination of the chemical constitution of plant beta-carotene and fish oil A_1. Figure 1 shows these structures and what happens when beta-carotene is converted into A_1. In Figure 2 are shown the chemical formulae of alpha and gamma carotene and cryptoxanthin.

Compare the formulae of the carotenes of Figure 2 and Figure 1 and it becomes evident that the ring compounds at the right and left of point (A) are alike in beta-carotene and different in alpha and gamma-carotene.

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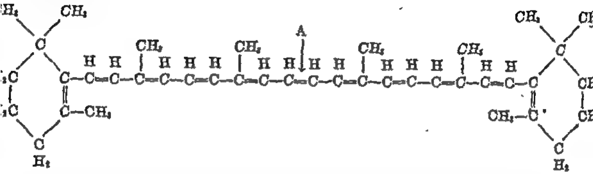
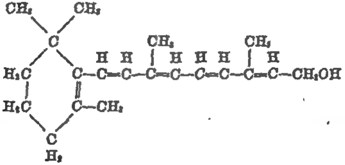
The left hand ring in all three is known as "optically inactive beta-ionone"

(see Fig. 2) and it must be present to produce active vitamin A. It was this fact and also the fact that beta-carotene contains two such rings while alpha and gamma carotene contain only one that led to the theory that beta-carotene's superior "A" activity was due to splitting into two molecules of A.



(a) Beta-Carotene formula:

Note that this formula, if broken in two at point A, would give two identically constructe olecules. In the liver this is accomplished by hydrolytic cleavage at point A, i.e., breakin addition of water. The result, 2 molecules of vitamin A from one molecule of beta-caroten iving the following formula are possible (see text).

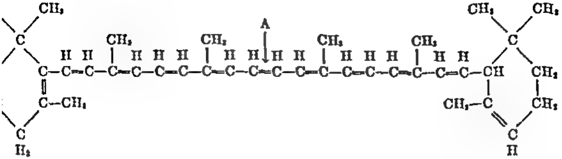


(b) Vitamin A formula:

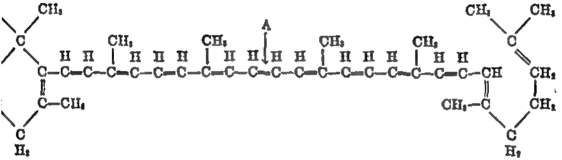
FIG. 1. WHAT HAPPENS WHEN BETA-CAROTENE BECOMES VITAMIN A

But as Clausen puts it: "A glance at the formulas of beta-carotene and of vitamin A shows that the transformation might occur (1) by hydration and splitting of one molecule of beta-carotene, to form two of vitamin A; or (2) by oxidation, to form one molecule of vitamin A. It was formerly believed that the first reaction occurred; because alpha and gamma carotene which contain but one beta-ionone ring appeared to be only half as active as beta-carotene in vitamin A activity. In repeating these experiments, Hickman and his colleagues have found no difference in the vitamin A activity of the three carotenoids. Moreover, by administering large doses of toc-

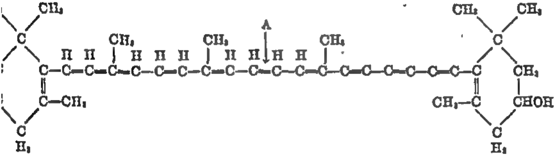
clinical support in the observation that persons with diabetic ketosis often have low concentrations of vitamin A in their blood, and high concentration of carotenoids."
(S. W. Clausen, Harvey Lectures. 1942–43).



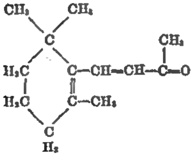
Alpha Carotene



Gamma Carotene



Cryptoxanthin



Optically inactive Beta-Ionone

FIG. 2. OTHER PROVITAMINS A

themselves or show their essentiality because the organism under test was actually synthesizing its requirement and until some means was found to cancel this synthetic operation, elimination of the factor from the diet failed to produce symptoms of deficiency. More of this phase of vitamin study later.

CHEMICAL NATURE OF VITAMIN B_1 (THIAMINE)

This vitamin has been described as the anti-beriberi vitamin, the antineuritic vitamin, aneurin in Europe and as B_1 or thiamine in America. It was first isolated in free form by Jansen and Donath in 1926. Funk's 1911 crystals which cured beriberi undoubtedly contained thiamine but were not pure.

By a modification of Jansen and Donath's procedure, R. R. Williams and associates increased Jansen and Donath's yields from rice polishings and ultimately established the structural chemistry. Williams and Cline succeeded in synthesizing the vitamin. Synthesis was accomplished almost simultaneously and independently in three other laboratories in 1937 (Andersag and Westphal at Elberfeld, Germany; Todd and Bergel at Edinburgh; Hoshimo and Ohta in Japan). Jansen called his product "Aneurin." The American Medical Association Council of Pharmacy adopted Williams' chemically descriptive name of *thiamin* or *thiamine.*

Figure 3 shows the structural formula deduced by R. R. Williams for the pure base and also its relation to co-carboxylase and the fluorescent product, thiochrome, which latter is produced and utilized in thiamine assay determinations.

Examination of Figure 3 shows justification of Funk in assigning the suffix "amine" to the anti-neuritic vitamin since thiamine contains the NH_2 group. Basically it is a union of two compounds, a pyrimidine base attached to a nitrogen-carbon-sulfur ring containing pentavalent nitrogen. This ring was tentatively identified by H. T. Clarke as a thiazole. Hence Williams' name of Thiamine or Sulfur Amine Compound. On treatment with sulfite, thiamine separates into two nuclei and this separation made its chemical identification possible. The reaction can also be accomplished with nitrite as follows:

$$C_{12}H_{18}N_4SOCl_2 + Na_2SO_3 \rightarrow \overset{\text{I}}{C_6H_9N_3SO_3} + \overset{\text{II}}{C_6H_9NSO} + \overset{\text{III}}{2\,NaCl}$$

Fraction I was proven to contain the pyrimidine nucleus.
Fraction II was shown by the collaboration of Clarke and Gurin to contain the thiazole nucleus.

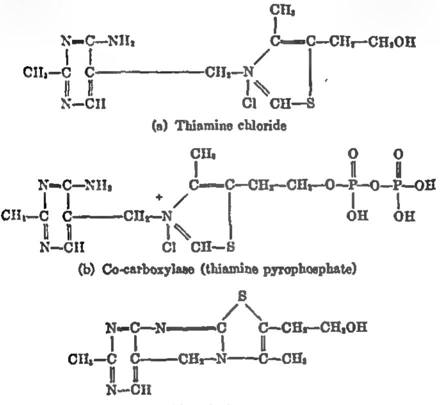
Some years ago Morgan showed that sulfuring of fruits reduced their B_1 content. The explanation is now clear. The sulfur dioxide uniting with

water formed sulfurous acid which actually destroyed the vitamin by cleavage as shown above.

In 1937 Löhman and Schuster isolated from yeast a crystalline substance which, in combination with an enzyme system containing the protein of washed yeast cells, de-carboxylated pyruvic acid, thus:

$$CH_3{-}CO{-}COOH \rightarrow CH_3{-}CHO + CO_2$$

They called this compound "co-carboxylase." It proved to be thiamine pyrophosphate. Thiamine is merchandized today as thiamine chloride. It



(a) Thiamine chloride

(b) Co-carboxylase (thiamine pyrophosphate)

(c) Thiochrome

FIG. 3. THIAMINE AND RELATED PRODUCTS

is destroyed by heat in neutral or alkaline solutions but pure thiamine in acid solution can be sterilized without loss of potency on heating 30 minutes at 120°C. It is comparatively stable to dry heat but it is common practice to include acids such as citric and tartatic in tablets and capsules as stabilizers of thiamine.

Vitamin B_1 crystals melt at 241°C. and leave no ash on ignition. They are hygroscopic and colorless with a slightly saline taste. An aqueous solution (1:20) of thiamine has a pH of about 3.5 and is optically inactive. In

aqueous solutions ranging from pH 3.5–5.0, it is quite stable. The isoelectric point of thiamine is between pH 9 and pH 10, nearer pH 9.

Kinnersley, O'Brien and Peters (1935) showed that blue-fluorescent oxidation products of pure B_1 are very slowly producible at room temperature by action of permanganate and manganese oxides at pH values more acid than pH 6, more rapidly at about pH 7. Kuhn, Wagner-Jauregg, Van Klaveren and Vetter (1935) isolated from yeast a yellow basic substance, $C_{12}H_{14}N_4OS$, whose neutral or alkaline solutions were characterized by intense blue fluorescence. They called this "thiochrome." (See Figure 3.) We know today that thiochrome can be formed from thiamine by a number of oxidizing agents and this reaction is the basis of one method of assay for thiamine potency.

The structure of thiamine pyrophosphate or cocarboxylase shown in Figure 3 was confirmed by Stern and Hofer (1938) who synthesized the compound by the action of phosphorus oxychloride on thiamine in the cold. Tauber (1938) phosphorylated synthetic thiamine to cocarboxylase by heating it with a mixture of sodium pyrophosphate and orthophosphoric acid at 155°C. for 15 minutes. Cocarboxylase is the active form of thiamine when it acts on pyruvates; phosphorylation apparently takes place in the tissues.

THE CHEMICAL NATURE OF VITAMIN B_2 (RIBOFLAVIN)

Vitamin B_2, or Vitamin G as it was first called, was originally believed to be the anti-pellagric vitamin. That view was definitely contradicted by Elvehjem and associates with proof that the pellagra preventive factor is nicotinic acid or niacin.

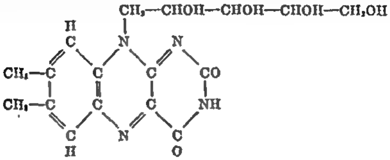
It was Kuhn and co-workers who first showed that vitamin B_2 belonged to a group of pigments called flavins and that these flavins were characterized by the presence of a pentose sugar known as d-ribose. Also that phosphorylated ribose-flavin was the prosthetic group of the respiratory enzyme described by Warburg and Christian as the "yellow ferment."

In 1933 Booher obtained from whey powder a pigmented substance which, when added to a basal diet devised by Bourquin and Sherman for use in demonstrating by rat growth vitamin G deficiency, proved growth stimulating.

It was in the same year that Kuhn and co-workers isolated from egg albumen a water-soluble green fluorescent pigment which, like Booher's pigment, stimulated the growth of rats on a Bourquin-Sherman basal diet in amounts as low as 0.1 milligrams per rat per day.

Kuhn's substance was not new to chemists. It had been isolated and partially described by Blyth in 1879 and by Bleyer and Kallman in 1925. It was Kuhn and co-workers, however, who demonstrated its "flavin"

structure, showed that it was an isoalloxazine derivative and obtained similar pigments or flavins from eggs, liver and milk to which were given at the time, the names ovo-flavin, hepato-flavin, and lacto-flavin. Later, however, these flavins were shown identical in structure. All contained d-ribose and so the source names lacto-, ovo-, and hepato-flavin were dropped and the substance called ribose-flavin or for euphony "riboflavin"; the present accepted name for vitamin B_2 or vitamin G.



THE STRUCTURE OF VITAMIN B_2 OR G (RIBOFLAVIN)

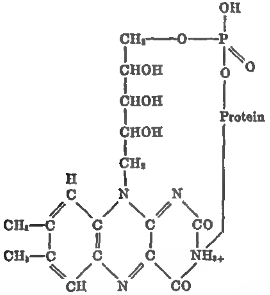


FIG. 4. THEORELL'S CONFIGURATION OF WARBURG'S YELLOW FERMENT

The chemical structure of riboflavin is shown in Figure 4. In Figure 4 is also shown Theorell's structure for Warburg and Christian's "yellow ferment."

Comparison of the two structures explains the discovery that phosphorylated riboflavin or Cytoflav was the prosthetic group in the yellow ferment, the specificity of the ferment being determined by the character of the protein to which the prosthetic group is attached.

This discovery was to have tremendous significance in blazing the trail to a new understanding of vitamin behavior, the role of some as prosthetic groups in respiratory enzyme systems.

In natural sources, riboflavin is found in both the free dialyzable state and in combination with various proteins.

Pure riboflavin crystals separate out from absolute alcohol solutions as yellow-orange colored needles. They have no sharp melting point but begin to darken at 240°C. and decompose at 274 to 282°C. They have a bitter taste. Molecular weight is about 376. They are soluble in water but insoluble in fat solvents.

Riboflavin develops a yellow-green fluorescence in neutral solutions; *maximum fluorescence at pH [illegible] to 7, diminishing as the pH shifts to either* the acid or alkaline side. This would indicate it to have an amphoteric character and *that the electrically neutral molecule is responsible for the* fluorescence.

In water solution and in acid solutions it is stable to heat but in alkaline solution it loses potency. It is also sensitive to both ordinary and ultraviolet light. It is not destroyed by oxidation or by ordinary oxidizing agents. Irradiation of alkaline solutions decomposes it and brings about the formation of a compound called lumiflavin ($C_{13}H_{12}N_4O_2$). Irradiation of acid solutions produces some lumiflavins and also an intensely blue fluorescent substance called lumichrome.

Steers fed a ration composed of corn, alfalfa, and a protein supplement have been shown able to synthesize riboflavin in the rumen but not if corn is omitted from the diet. Riboflavin is now prepared in the laboratory by synthesis but we have no evidence that man can synthesize it in his body.

THE CHEMICAL NATURE OF NIACIN

The first steps in the resolution of the vitamin B Complex showed that pellagra was neither prevented or cured by the vitamin that cured beriberi; was not thiamine deficiency. In 1937, Elvehjem and co-workers demonstrated that the characteristic lesions of both blacktongue in dogs and probably human pellagra were *preventable and cured by a substance* known to chemists since *1867 (Huber) and called nicotinic acid.* In the same year, (1937), Fouts, Harris, France, Spies, and Sebrell independently showed its *specificity* for human pellagra.

In the years 1911–12, Funk in reporting the isolation of an anti-beriberi factor from rice polishings reported the recovery of two crystalline products:

$C_{26}H_{20}N_4O_6$; M.P. 233°C.
$C_6H_5O_2N$ (Nicotinic acid)

and extraction of 4 crystalline products from yeast described as follows (1913):

(a) Crude crystalline product . . . . cura. of beriberi
(b) $C_{24}H_{18}O_{6}N_{4}$; M.P. 229°C. . . . . improved beriberi cases
(c) $C_{6}H_{4}O_{2}N$ (nicotinic acid); M.P. 235°C . . . . . no activity
(d) $C_{18}H_{22}O_{6}N_{4}$; M.P. 222°C. . . . . no activity

But that better results were obtained by combination of fractions (b), (c), and (d) than with (a) alone.

These findings of Funk in 1913 are particularly interesting today with our better knowledge of the pellagra syndrome. While niacin is now recognized and proven specific against the most characteristic lesions of pellagra, it is also true that the disease as it occurs in man is usually a multiple vitamin deficiency and involves secondary deficiencies benefitted by thiamine and riboflavin and other members of the B Complex. Also, that while thiamine is the specific for beriberi its action in the respiratory enzyme system also involves the presence of other coenzyme materials in which niacin forms a significant group (see Fig. 5).

The data given by Funk in 1913 are also of special interest in that it has sometimes been stated that Funk's anti-beriberi crystals were contaminated by nicotinic acid. Funk at the time definitely recognized nicotinic acid as a separate constituent of rice polish and yeast and that it augmented what we now call thiamine reaction. He did not pursue its action farther at the time as he was then primarily concerned with a specific for beriberi.

Because the public confused nicotinic acid with nicotine, it seemed desirable to coin a new name for the product and *niacin* was selected and approved by the American Medical Association's Council of Pharmacy. Nicotinic acid can be produced from nicotine in the laboratory by oxidation but the human system can not accomplish this oxidation. Nicotine is not anti-pellagric.

Niacin or nicotinic acid is a derivative of pyridine. The structure of niacin, niacinamide and coramine, the three forms sufficiently potent to use on pellagra patients, and their relation to pyridine and other pyridine derivatives is shown in Figure 5.

Niacin in the pure state consists of white, odorless needles or powder with a slightly bitter taste. It is sparingly soluble in water (about 1 part in 100 parts of water); readily soluble in alcohol and in solutions of alkali carbonates; soluble in glycerol but insoluble in ether. Its crystals melt at 234° to 236°C. It acts, chemically, as a weak acid and readily forms sodium and potassium salts which have a mild alkaline reaction. According to Perlzweig, nicotinic acid is metabolized and excreted mainly as trigonelline (see Fig. 5) and nicotinuric acid.

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$C_{26}H_{20}N_4O_9$; M P. 233°C.

$C_6H_5O_2N$ (Nicotinic acid)

Singal and Sydenstricker have reported that pyridoxine, like niacin, appears to influence the concentration of Najjar's F_1 fluorescent substance in the urine.

THE CHEMICAL NATURE OF PANTOTHENIC ACID OR PANTOTHEN

The discovery and isolation of this vitamin (sometimes called the anti-gray hair factor) was a result of the study of factors (R. J. Williams' "nutrilites") required for the growth of yeast. Its relation to animal nutrition was established when Lepkovsky and Jukes showed it curative of a type of chick dermatitis that responded to treatment with what was first called Filtrate Factor II. In 1936, Lepkovsky, Jukes and Krause established that if from an extract of liver one removed B_1 and B_2, the resulting filtrate contained factors curative of certain dermatoses in rats, dogs and

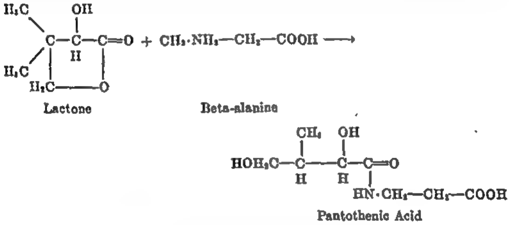

FIG. 7. PANTOTHENIC ACID (PANTOTHEN)

chicks. The isolation of niacin identified one of these filtrate factors. The isolation of B_6 from Filtrate Factor I removed another. Pantothenic acid proved to be in Filtrate Factor II. The original Filtrate Factor II, then, contains pantothenic acid but it may contain other nutrilites. The term "Filtrate Factor II" is, therefore, *not identical* with pantothenic acid. Its achromotrichial activity was first shown by Morgan who proved that deficiency of Filtrate Factor II in rat diets produced greying of hair and that under certain conditions this could be prevented and cured by pantothenic acid or its stable salt, calcium pantothenate.

The structural formula of pantothenic acid and its relation to beta-alanine is shown in Figure 7. The empirical formula for the calcium salt is $Ca(C_9H_{16}O_5N)_2$. One of the cleavage products was proven to be beta-alanine. Pantothenic acid was proven to be stimulatory to yeast in concentrations as low as 0.0000008 milligrams per ml. of culture medium.

THE CHEMICAL NATURE OF VITAMIN B_6 (PYRIDOXINE)

A specific type of rat skin lesion attended by a pink or florid dermatitis (acrodynia) permitted the differentiation of another vitamin of the vitamin B Complex. This was first described as Filtrate Factor I (Lepkovsky, Jukes and Krause) and is apparently identical with Booher's and Hogan and Richardson's "H" factor but not the vitamin H of György and Parsons. It is the "Y" factor of Chick and Copping.

The vitamin was isolated *and chemically identified in six laboratories in* the same year (by Lepkovsky, György, Kuhn, Ichiba, Emerson, Keresztesy) Keresztesy and Stevens in the Merck Laboratory reported its empirical formula to be $C_8H_{11}NO_3$ and Harris and Folkers in the same laboratory, confirmed this by synthesizing the compound.

Kuhn and Wendt suggested the name *Adermin* but later, György and Eckhardt suggested the present accepted name of *Pyridoxine.* Like niacin, pyridoxine is a pyridine derivative. For structure see Figure 6.

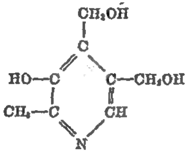

FIG. 6. VITAMIN B_6 (ADERMIN OR PYRIDOXIN)

Pure pyridoxine consists of white platelets which melt with decomposition at 206–208°C. One gram of the hydrochloride is soluble in 4.5 ml. of water and in 90 ml. of alcohol. Aqueous solutions have a pH of 3.2.

Pyridoxine is stable to concentrated HCl at high temperatures, and is not affected by heating with alkalies, ethyl nitrate, nitrous acid or Fehling solution. It is destroyed by light and by ultraviolet irradiation.

It gives an orange-yellow color with ferric chloride and with diazotized sulfanilic acid.

Kuhn found it impossible to dialize it from yeast indicating that it may be present in combination with protein and perhaps, *like thiamine,* riboflavin, and niacin, form part of a respiratory enzyme system.

Birch, György, and Harris first related rat acrodynia to pyridoxine deficiency and at first B_6 potency of foods was expressed in biological units (György; Wilson and Roy; Schneider, et al; Quackenbush). György's and Wilson and Roy's unit was the amount necessary to cure rat acrodynia in 2 weeks or 0.1 milligrams of pure pyridoxine.

A deep yellow color results on addition of p-methyl amino benzaldehyde in glacial acetic acid to para-amino-benzoic in glacial acetic which can be measured colorimetrically and used in assay procedures.

It is claimed that Paba modifies the type of melanin produced from the oxidation of dopa by dopa oxidase. It also retards the aerobic oxidation of tyrosine and accelerates the oxidation of para-cresol. Qualitively in these respects it has the same influence as aniline but quantitively the effect is greater than that of aniline.

THE CHEMICAL NATURE OF INOSITOL

In 1928 Eastcott obtained from tea a substance which stimulated yeast growth and which proved to be a phytin derivative known as "inositol." This put inositol in the group of "bioses" or yeast nutrilites.

In 1940 Wooley reported that the mouse requires a vitamin for normal growth and maintenance of hair. This anti-alopecia factor was proven to

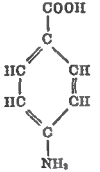

FIG. 8. PARA-AMINOBENZOIC ACID (PABA)

be inositol. Phytin, the Ca-Mg salt of inositol phosphoric acid was effective but phosphorylation was not essential as inositol itself allowed this anti-alopecia activity and weight increase effect.

The chemical structure of inositol and phytin are shown in Figure 9.

Inositol or Inosite is not a sugar though it has the same empirical formula as glucose ($C_6H_{12}O_6$). It is an alcohol, not an aldehyde. It crystallizes in large, rhombic crystals having a melting point of 225°C. It is soluble in water, 1 part in 7.5 parts of water, insoluble in strong alcohol and in ether.

THE CHEMICAL NATURE OF BIOTIN (VITAMIN H; COENZYME R)

In 1922 Fulmer and Nelson postulated that the "bios" of Wildier was not a single substance. Lucas (1924) suggested the terms Bios I and Bios II for the fractions. Later Bios II was further subdivided into Bios II A and Bios II B. (Miller and Eastcott et al 1932 and 1933.) We have already noted that Williams and Truesdail demonstrated that pantothenic acid

Potency is greatest in presence of asparagine or aspartic acid in the culture medium.

Pantothenic acid itself is quite unstable. Its synthesis was announced by Williams and Major in 1940 as the result of isolation and later, the synthesis of the previously unknown cleavage product as a pure crystalline material, 1-l-hydroxy-β,β,-dimethyl-γ-butyrolactone by Stiller, Keresztesy and Finkelstein (1940). It was subsequently synthesized by Stiller, Harris, Finkelstein, Keresztesy and Folkers in the same year by condensation of the synthetic cleavage product with Beta-alanine.

The salt of pantothenic acid, calcium pantothenate, is the form found in capsules and tablets today. It is a white, odorless, crystalline powder with a slightly bitter taste. One gram dissolves in about 7 ml. of water. It is insoluble in alcohol. The pH value of a solution protected against CO_2 is 8.72. Molecular weight is 476 54. Ca-pantothenate loses activity on autoclaving and sterilization must be accomplished by Berkefeld or Seitz filtration. It is stable to light and air; not hygroscopic except under conditions of excessive humidity.

R. J. Williams has suggested the shorter name of "Pantothen" be used to designate this vitamin.

THE CHEMICAL NATURE OF PARA-AMINO BENZOIC ACID (P.A.B.) (PABA)

Para-amino benzoic acid was isolated and synthesized by Ansbacher and reported in February 1941 (Science 93: 164, 1941). In this report, Ansbacher stated:

> "About a year ago Woods and Fildes (1940) reported the anti-sulfanilimide activity in vitro of p-amino benzoic acid In April (1940), Woods found that yeast extracts contain a substance which nullifies the inhibitory action of sulfanilamide on the growth of hemolytic streptococci, and presented circumstantial evidence that the yeast factor may be p-amino benzoic acid. In December, Rubbo and Gillespie recovered p-amino benzoic acid as the benzoyl derivative from yeast and concluded it to be a bacterial growth factor Experiments conducted in this institute indicate that para-amino benzoic acid, considered to be an essential metabolite by Fildes (1940) is a vitamin, namely a chromotrichia factor for the rat and a growth promoting factor for the chick.

It is now proven that para-amino benzoic acid is a B Complex vitamin, a natural constituent of yeast, a growth factor for chicks, an essential nutrilite for bacteria, and has anti-gray hair activity in the nutritional and hydroquinone achromotrichia of the rat. Ansbacher has suggested for it the shorter term "PABA."

The structure of para-amino benzoic acid is shown in Figure 8.

Paba occurs as a colorless amorphous powder or in needle-shaped crystals, has a molecular weight of 137 and a melting point of 186–187°C.

A deep yellow color results on addition of p-methyl amino benzaldehyde in glacial acetic acid to para-amino-benzoic in glacial acetic which can be measured colorimetrically and used in assay procedures.

It is claimed that Paba modifies the type of melanin produced from the oxidation of dopa by dopa oxidase. It also retards the aerobic oxidation of tyrosine and accelerates the oxidation of para-cresol. Qualitively in these respects it has the same influence as aniline but quantitively the effect is greater than that of aniline.

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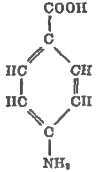

FIG 8. PARA-AMINOBENZOIC ACID (PABA)

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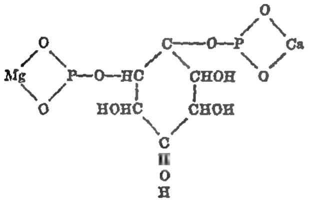
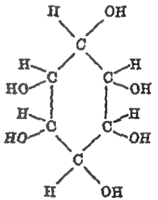
was one of these "bios" fractions. In 1936 Kögl and Tönnis isolated biotin from fraction Bios II B, obtaining it from crystalline egg yolk. Their isolation of biotin, therefore, was a direct consequence of search for a "bios" factor.

Meanwhile, Allison, Hoover and Burk (1933) concentrated a nutrilite for the root nodule bacterium *Rhizobium* from hydrolyzed yeast, commercial molasses and sucrose. It had a marked effect on *Rhizobium* respiration and they called it Coenzyme R. It was later shown by West and Wilson (1939, 1940) to be identical with Kögl and Tönnis' biotin.

In 1927 Boas described an injury induced in rats by feeding dessicated egg white as a protein source. She also showed that egg white cooked before drying did not have this effect; that certain foods possessed a factor which counteracted this toxic effect and postulated in them a protective



Phytin



Inositol

FIG. 9. PHYTIN AND INOSITOL

factor "X." This egg white injury was further studied between 1931 and 1940 by Parsons and Kelly (1933) and by P. György (1931 and 1940). György concentrated the factor that corrected this egg white injury and suggested for it (1931) the name vitamin H; H being selected as the initial of the German word "Haut" or skin, and because the substance cured the egg white dermatitis. The identity of vitamin H and biotin was established by György, Rose, Hoffman, Melville, and duVigneaud in 1940.

The substance in dessicated raw egg white that neutralized its biotin content and created a biotin deficiency was proven to be a protein-like substance for which the name *avidin* has been selected. Cooking destroys this avidin or, at least, renders it incapable of inactivating biotin. Egg white injury has been produced in man and cured by biotin according to Sydenstricker et al (1942).

Crystalline biotin was first isolated from duck egg yolk in the form of its

methyl ester by Kögl and Tonnis (1936). Conversion of the methyl ester to free biotin was effected by hydrolysis with HCl or NaOH (1941). The structural formula assigned to biotin obtained by total laboratory synthesis as reported by Harris, Wolf, Mozingo and Folkers confirmed the conclusions drawn by duVigneaud and co-workers and is shown in Figure 10. It is the second vitamin shown to contain the element sulfur (thiamine was the first).

Pure biotin and biotin methyl ester occur as colorless, crystalline solids. Biotin crystallizes from water in long thin needles; biotin methyl ester from a methanol-ether solution in long, thin, platelike needles. Melting points for biotin crystals have been reported at 216° and 230–232° varying with the source. Similar discrepancy appears in reports of the methyl ester crystals at 161°C. and 166–167°C.

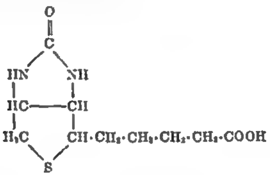


FIG. 10. BIOTIN

Biotin is optically active; specific rotation $(\alpha)_D^{22} = +92°$ for a 0.3 per cent solution in 0.1 N NaOH. The methyl ester has a specific rotation of $(\alpha)_D^{22} = +57°$ for a 1 per cent solution in chloroform and $(\alpha)_D^{15} = +82°$ for a 0 59 per cent solution in methyl alcohol.

Biotin is relatively insoluble in water, crystallizing readily from it. In basic solutions it is readily soluble; only slightly soluble in weak acids. It is insoluble in ordinary organic solvents but the methyl ester is appreciably soluble in chloroform, alcohols, ketones, and halogenated hydrocarbons.

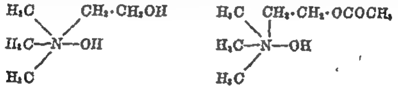
Prolonged aeration of biotin in acid or alkaline solution with air or oxygen does not destroy its activity but stronger oxidizing agents (e.g. H_2O_2) quickly destroy activity. Nitrous acid destroys activity but ninhydrin does not, demonstrating that it is not an alpha-amino acid. Activity is not destroyed by acetylation, alkylation or carbonyl reagents.

Resynthesis of biotin from its degradation product (diaminocarboxylic acid) by treatment with phosgene supported the idea that it contains a cyclic urea ring (1941).

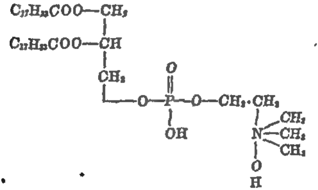
THE CHEMICAL NATURE OF CHOLINE

Choline as a group in the phospholipid lecithin has been known for many years. In the combination known as acetylcholine it was known to function in the transmission of nerve impulses across peripheral synapses and motor nerve endings.

Its inclusion in the vitamin B Complex came with the discovery of its lipotropic action. Rats on a low choline diet develop fatty livers. They also develop a hemorrhagic degeneration of the kidneys. Diets low in choline, therefore, like diets low in vitamins such as thiamine, vitamin D,



Choline *Acetylcholine*



Dioleyllecithin

FIG. 11. CHOLINE AND CHOLINE COMPOUND

and vitamin C induce pathologic conditions which appears to justify consideration of choline as a vitamin.

The structure of choline and its form in lecithin and acetylcholine are shown in Figure 11.

It is a colorless, viscid, strongly basic liquid, very soluble in water and insoluble in ether. It readily absorbs CO_2 from the air. For therapeutic use the chloride is used. The chloride is in the form of colorless, deliquescent crystals, very soluble in water or alcohol.

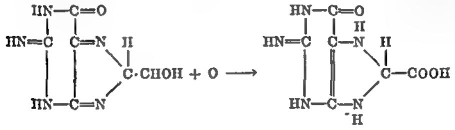
Acetylcholine chloride is a white, odorless, hygroscopic powder, very soluble in water or alcohol; insoluble in ether.

Lecithin is a brownish-yellow, waxy, hygroscopic product which swells in water and in NaCl, forming a colloidal solution. The transformation

of fatty acids to lecithin by combination with phosphoric acid and choline permits their passage from the liver into the blood. As Bloor puts it: "In view of the fact that choline is an important constituent of lecithin, and since lecithin appears to be a primary step in fat metabolism, the conception arises that fatty livers are the result of inability to form lecithin fast enough to take care of a large amount of fat, which in turn is caused by a deficiency of choline." . . . "Choline may be either a catalyst or a material essential to phospholipid formation."

THE CHEMICAL NATURE OF FOLIC ACID

In 1941 Mitchell, Snell and Williams reported that using *streptococcus lactis R* as a test organism they obtained in nearly pure form from spinach



Xanthopterin Leucopterin

FIG. 12. XANTHOPTERIN

an acid nutrilite. The concentrate contained no nitrogen, sulfur or phosphorus and had a molecular weight of about 500. They stated:

"This acid, or one with similar chemical and physiological properties, occurs in a number of animal tissues of which liver and kidney are best sources. It is widespread in the biological kingdom. Mushrooms and yeast are good sources. It is especially abundant in green leaves of many kinds, including grass. Because of this fact, and since we have obtained what appears to be a nearly pure chemical entity, we suggest the name "folic acid (Latin, folium-leaf)". . . "Folic acid stimulates the growth of L. casei under the same conditions as the factor reported by Snell and Peterson (1940) and recently reported to be isolated by Stokstad (1941)."

The product was adsorbable on charcoal.

In 1940 Snell and Peterson reported what they called an eluate factor necessary for the growth of *L. casei*. At first it appeared that folic acid and Snell and Peterson's *L. casei* eluate factor might be identical but subsequent studies have raised doubts as to whether Mitchell, Snell, and Williams' folic acid concentrate is a single nutrilite or a combination of several different nutrilites.

There is evidence that Factor U, Hogan and Parrott's B_c, Day's Vitamin M may be identical with the *L. casei* eluate factor and that Elvehjem's

10 and B_{11} and a factor described by Keresztesy et al. are members of the lic acid complex.

For discussion of the present status of folic acid see Chapter X.

THE CHEMICAL NATURE OF VITAMIN C

The discovery that lemon juice contains a substance corrective of scurvy es back to the 1700's. The elucidation of the chemical nature of the ti-scorbutic substance, vitamin C, is the product of studies in many boratories. In 1932, King and Waugh obtained from lemon juice an tively anti-scorbutic substance, apparently identical chemically with the lexuronic acid" recovered from adrenal cortex and from cabbage and anges by Szent-Gyorgyi. The chemical structure of this substance was tablished in 1933 by Haworth, Hirst and collaborators and this structure as confirmed by synthesis of the product by Reichstein, Grussner, and ppenhauer in the same year (1933–34).

The outstanding characteristic of vitamin C or ascorbic acid, as it is now lled, is that it is reversibly oxidizable. For structure of the commonly curring l-ascorbic acid form and its oxidation products, see Figure 13.

There are other forms of ascorbic acid which have some potency—for ample, l-rhamno-ascorbic acid and l-araboascorbic acid but these are uch less potent than the "l" form. It would appear from a study of ese modifications of the ascorbic acid structure that one essential to anti-orbutic activity is associated with the position of the oxygen ring; the l" forms being inactive, the "l" forms active. However, the ring position n not be the only factor concerned with physiological activity because e two "l" forms, named above, have the ring in the proper position but hibit only $\frac{1}{5}$th to $\frac{1}{20}$th the potency of the l-ascorbic acid form.

Its chemical formula shows it to be a sugar acid which indicates a rela-nship to carbohydrates from which the vitamin is probably derived in logic systems.

Ascorbic acid occurs as an odorless, white or slightly yellow, crystalline oduct which slowly darkens on exposure to light. The melting point the product is 189°–192°C. One gram dissolves in about 3 ml. of water, ml. of alcohol, 100 ml. of glycerol. It is insoluble in benzene, chloro-m, ether, petroleum ether, and fats.

In the dry form ascorbic acid is quite stable and it is more stable in id solutions than in the presence of alkali. It is optically active and ly the levorotary forms have vitamin activity. Protection against struction requires prevention of oxidation. It is not destroyed by heat one but in the presence of oxygen or oxidizing factors, the rate of destruc-n proceeds faster with rise in temperature. From a practical viewpoint is means that products containing ascorbic acid and exposed to air will tain their anti-scorbutic activity better if kept cold.

As shown in Figure 14, oxidation proceeds in two steps. The first step, namely conversion to dehydroascorbic acid, is reversible, which means that in assaying for ascorbic acid content the test must measure content of both forms as the body can utilize either. If oxidation is prolonged the dehydro form breaks down farther as shown and this step is not reversible.

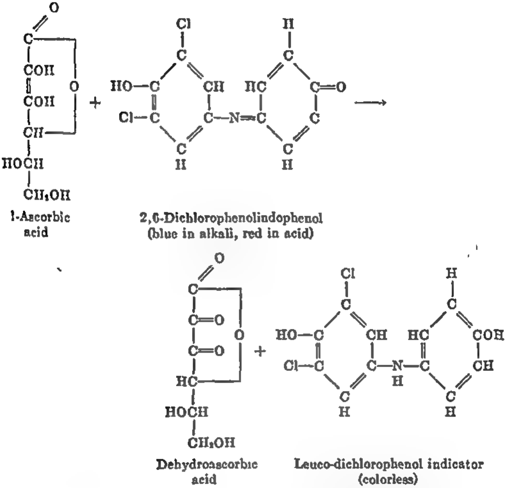
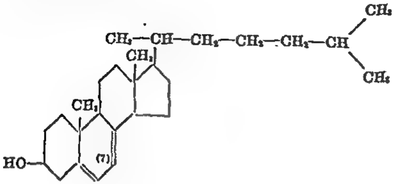


Fig. 13 Reaction of Ascorbic Acid with Indicator Dye
(After Bessey, J. A. M. A. 111. 1290, 1938)

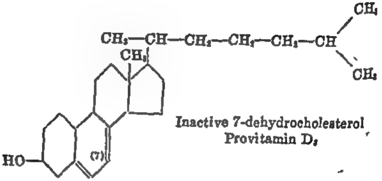
THE CHEMICAL NATURE OF VITAMIN D

Vitamin D, like vitamin A, belongs to the so-called fat soluble vitamins. The search for a substance corrective of rickets has led to date to the postulation (Bills) of at least 10 compounds of a sterol type having rickets-healing potency. With one exception, all of these substances require ultraviolet irradiation to convert them from the physiologically inactive provitamin D to the cure-effecting active vitamin D.

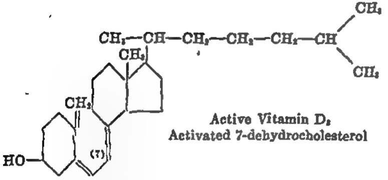
other fungi and is the form present in the milk of cows when these cows have been fed irradiated yeast. Activated ergosterol or calicferol dissolved in an inert oil is called "viosterol."



Ordinary cholesterol.
Not antirachitic



Inactive 7-dehydrocholesterol
Provitamin D_3



Active Vitamin D_3
Activated 7-dehydrocholesterol

FIG. 17. RELATION OF CHOLESTEROL TO VITAMIN D_3 (7-DEHYDROCHOLESTEROL)

Calciferol is also called "vitamin D_2," and activated 7-dehydrocholesterol is called "D_3." There is no vitamin D_1 in the literature today, the compound first called by that name by German workers having proved to be a mixture of calciferol and an irradiation product known as lumisterol.

When pure ergosterol is irradiated to convert it into calciferol, ordinarily not more than 50 per cent is changed to calciferol under ideal conditions.

During the processes of oxidation a series of compounds are formed which, in the order of their appearance, are listed by Bills as follows:

(1) Ergosterol
(2) Lumisterol
(3) Tachysterol
(4) Calciferol
(5) Toxisterol (Substance 248)
(6) Suprasterols I and II

From such oxidation mixtures, lumisterol, the two suprasterols and calciferol have been isolated in crystalline state. Tachysterol has been separated as a benzoate. Toxisterol has not been isolated in pure state. It gets the name "Substance 248" because it has an absorption band at 248 $\mu\mu$. Of all of these compounds only calciferol has antirachitic action. Lumisterol can be converted to calciferol and may form with it an additional compound consisting of 1 part lumisterol to 1 part of calciferol. It was this addition product that German workers originally classed as vitamin D_1.

Toxisterol, as the name suggests, may produce a toxic effect. Toxisterol, tachysterol, and the suprasterols are non-antirachitic but the latter two are less toxic than toxisterol. The toxic effect sometimes obtained in the past with irradiated ergosterol preparations is now believed to be due to failure to eliminate the toxisterols and tachysterols.

Both vitamin D_2 and D_3 are insoluble in water but soluble in oil, fats, and fat solvents

A product known as 7-dehydrocampesterol, with a potency of 4,000,000 units per gram has been recently reported and some interest attaches to a product called AT-10 which Shohl and Farber state to have 1/400th the potency of D_2. It is believed that the principal component is di-hydro-tachysterol.

THE CHEMICAL NATURE OF VITAMIN E

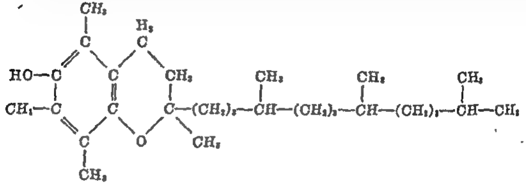
Vitamin E was first called "X" by its discoverers, Evans and Bishop. When it was proved that the substance was a true vitamin, Sure suggested that it was entitled to be called vitamin E.

Evans showed that one of the most abundant sources of this anti-sterility vitamin was wheat germ oil and wheat germ oil has been the base from which products of vitamin E character have been isolated.

In 1936, Evans et al. isolated substances with vitamin E properties and gave them the names of alpha and beta-tocopherol. Fernholz suggested for alpha-tocopherol the formula shown in Figure 18 and this structure has been confirmed by synthesis carried by out Karrer and his collaborators. This is the most potent form obtained to date; beta-tocopherol and gamma-tocopherol being slightly less active than alpha-tocopherol.

As shown by the formula, alpha-tocopherol is a chroman derivative (see Fig. 18). Alpha-tocopherol is a light yellow, odorless, viscous, oily liquid with an insipid taste and a molecular weight of 430. It decomposes on boiling at atmospheric pressure. It has a specific gravity of 0.95 at 25°C. It is insoluble in water but soluble in fat and fat solvents.

Alpha-tocopherol is stable to heat, alkalies, and acids. It is readily destroyed by rancid fats. It is a strong reducing agent and therefore readily oxidized by oxidizing agents and the salts of heavy metals. Because of



α-Tocopherol acc. to Fernholz (1938); Chromane nucleus

FIG. 18. FORM OF VITAMIN E

TABLE IV

Dose Equivalents of Alpha-tocopherol

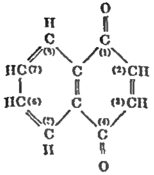
	DOSE EQUIVALENT TO 1 MGM. NATURAL ALPHA-TOCOPHEROL
	mg
1. Natural alpha-tocopherol	1.0
2. Alpha-tocopherol acid succinate	1.0
3. Beta-tocopherol	2 5
4. Beta-tocopherol azo-benzene-4-carboxylate	8.0
5. Gamma-tocopherol	12.0
6. Gamma-tocopherol palmitate	12.0
7 Synthetic dl-alpha-tocopherol	1.3

this readily oxidizable character, it is extensively used today in vitamin mixtures to protect other oxidizable vitamins from destruction. In other words, it is present as an antioxidant in many mixtures rather than for its anti-sterility values.

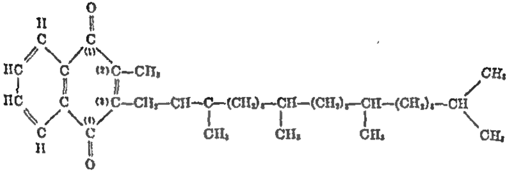
For relative potencies of the tocopherols see Table IV.

THE CHEMICAL NATURE OF VITAMIN K

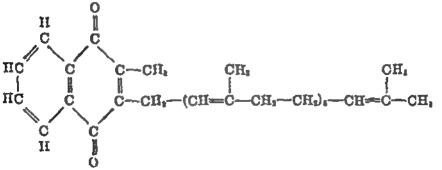
In 1929 Dam described a hemorrhagic disea[illegible] characterized by a prolonged clotting time. In 1935 he chara[illegible] condition as due



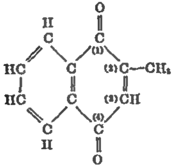
(a) 1,4 Naphthoquinone



(b) Natural vitamin K_1; 2-methyl-3-phytyl-1,4-naphthoquinone



(c) K_2



(d) Menadione; 2-methyl-1,4-naphthoquinone

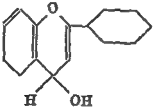
FIG. 19. RELATIONSHIP OF NATURAL VITAMINS K TO MENADIONE

to deficiency of a "Koagulation Vitamin" or Vitamin K, present in leafy vegetables and in hog liver fat.

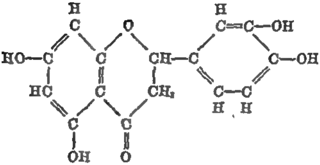
The elucidation of the chemical structure of vitamin K or vitamins K has been amazingly rapid. The significant nucleus of all these vitamins is the chemical compound known as naphthoquinone. Two forms of the natural vitamin K have been obtained, K_1 from alfalfa and K_2 from putrid sardine meal. They were found in the nonsterol fraction of the non-

Flavanol structure

I.

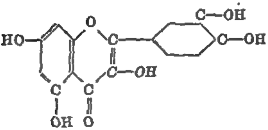


II.



Eriodictyol

III.



Quercitin

FIG. 20. STRUCTURE OF ERIODICTYOL (VITAMIN P)

saponifiable fraction of the concentrate from these sources. The chemical structures of K_1 and K_2 are shown in Figure 19.

Further study of compounds with the naphthoquinone nucleus showed that 2-methyl-1,4-naphthoquinone is actually more potent than the natural vitamins K. It is this compound that is today known as *menadione*. For structure, see Figure 19. Menadione is a slightly yellowish crystalline material rapidly destroyed by alkalies, sunlight, oxidizing agents, strong

acids, and aluminum chloride. It is stable to heat and reducing agents and is not water soluble. Bile is necessary for its physiological absorption.

Vitamin K is now known essential to the formation of prothrombin but is not a part of the prothrombin molecule.

Anderson and Newman first isolated a substance called phthiocol from the lipids of *M. tuberculosis.* This compound has about 1/500th the activity of vitamin K_1. Apparently, the CH_3 group in position 2 is essential to activity.

THE CHEMICAL NATURE OF VITAMIN P (PERMEABILITY VITAMIN)

One function of ascorbic acid is to prevent capillary fragility. In treating certain cases with tendency of capillaries to bleed (hemorrhagic diathesis), it was discovered that while this tendency could be checked with l-ascorbic acid, certain natural vegetable juices, notably paprika juice, showed superiority over the use of the synthetic vitamin C alone. The observers sought for another hemorrhage controlling factor and believed they had found it in a flavonol fraction to which they gave the name "Vitamin P." As the discoverer, Szent-Gyorgyi, states in his record:

> "I called it Vitamin P in honor of paprika and permeability on which later it was found to have an influence"

The active fraction from lemon juice containing this factor has been called "citrine." Lorenz and Arnold state that the water extract of a whole lemon contains 1.7 mgm. of citrine flavonols per gram of lemon but emphasize that the citrine fraction is a complex containing several compounds, not all of which are active.

The structure shown in Figure 20 has been suggested for the active vitamin P and for it, the name "eriodictin or eriodictyol."

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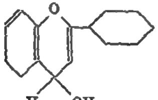
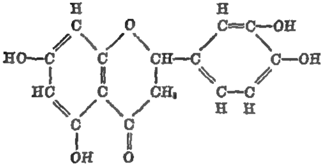


II.



Eriodictyol

III.

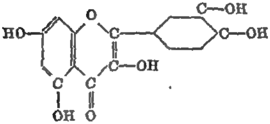


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Fig. 20. Structure of Eriodictyol (Vitamin P)

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Chapter III

VITAMIN BEHAVIOR

In 1911, when Casimir Funk first coined the name *vitamine*, intere was mainly in the prevention of disease; the particular disease known beriberi or polyneuritis. But, by 1913, Funk had reached the conceptic of a *group of substances* concerned in prevention of what he then describe for the first time, as the *avitaminoses*, and predicted that these woul include scurvy, rickets, Barlow's disease, pellagra, and sprue.

As early as 1906 Sir Gowland Hopkins suggested the existence of wh he called "accessory factors" which might play a role in *normal* nutritio Osborne and Mendel's experiments with protein-free milk and McCollum recovery of a non-amine growth factor from butter and egg yolk widene the interest in what we now call vitamins from their relation to specifi diseases to their possible concern in the control of normal behavior. Th concept brought into the study of vitamins and avitaminoses the bic chemists and physiologists who were trying to explain physiologic behavior; to determine the scientific basis of nutrition. Vitamins were n longer simply drugs with which to treat disease but, like proteins, fats, an carbohydrates, were to be considered as nutrients; needed by the sick an well.

Owing to slow progress in the isolation, chemical identification an synthesis of vitamins, animal behavior was for some time our sole mean of proof of vitamin existence. We knew of sources of vitamins A, B, and C for years before any one ever saw the pure substances or knew their chemica structure. We called them by names that described their function, suc as the anti-scorbutic factor, the anti-beriberi factor, the antineuritic factor etc., or by letters—vitamins A, B, C, D, etc.

As the study of these substances progressed farther it became eviden that while vitamins were present in both plant and animal tissues thei primal origin was in plants. But the plant physiologists were, with some notable exceptions, slow to concern themselves with either how or why the plants made these products. One notable exception was Wildier's postulation of a bios for yeast in 1901. The search for its nature is responsible for the unravelling of many of the components of the vitamin B complex. In fact, the first comprehensive review of the relation of plants to vitamin synthesis and their significance to the plant parent has only just appeared (Schopfer's "Plants and Vitamins," 1943). With pure and synthetic vitamins to use, these studies of vitamins in plants have provided another

significant contribution to our modern concept of the "how" of vitamin behavior.

PLANT ACTIVATORS (WIRKSTOFFE)

Wildier's 1901 postulate that yeasts require for growth a substance of organic nature different from ordinary nutrients known at the time, and effective in microscopic amounts can probably claim priority in the study of plant growth activators.

Between 1914 and 1919 Bottomley and Mockeridge first suggested that green plants require something similar in action and chemical nature for

TABLE V

Classification of Active Substance

(Wirkstoffe) acc to Schopfer

Group I.	Pseudo-growth factors of a mineral nature
	Includes mineral substances such as Zn, Mn, Mo, Cu with catalytic and oligodynamic action Some able to function as co-factors of growth or act in conjunction with co-factors in enzyme systems
Group II.	Growth factors of a vitamin nature, acting on metabolism or on the growth of cytoplasm
	Includes all the vitamins whose action has been demonstrated in plants; the "B" substances of Nielsen, some of the "BP" substances of Euler, the "Z" fermentation factor; the vitazymes in coenzymes, the bios substances
Group III.	Factors of a hormonal nature acting in a specific manner on the morphology of the organism
	Includes mainly the "Auxin" group; Nielsen's "A" substances

N B *Schopfer's definition of a vitamin:*

"*Vitamin factor is an organic substance, the need for which results from* the loss of the capacity for synthesis, whose action is catalytic (i.e., active in small amounts), quantitative, and markedly specific "

growth, and suggested for those substances the name "auximones"; since shortened to "auxins."

From these beginnings has grown up a classification of plant growth activators which Schopfer tabulates as follows (see Table V).

Nielsen defined his "B" substances as *water*-soluble substances acting as growth factors for microorganisms. He called "A" substances, *ether*-soluble substances acting as hormones.

von Euler's "BP" substances he defined as *all* water-soluble factors required by plants. He also coined the term "Ergones" to embrace vitamins and hormones and Ammon and Dirschel use the term "ergines" to include ergones and enzymes. This nomenclature of plant activities is of special interest because the terms coined and the groups listed indicate

the progress that has been made toward understanding of their role in maintenance of life and growth and the attempt to express it in nomenclature.

We shall return later to specific action of vitamins in the behavior of plants and the reasons for their synthesis by plants and in certain regions of the plant body.

THE RELATION OF VITAMINS TO ENZYMES AND COENZYMES

In modern nomenclature these terms are used to describe the chemical *structure and parts of enzymes.*

Enzyme or Holoenzyme = apo-enzyme + coenzyme. By *apoenzyme* is meant the colloidal protein "Carrier" (Träger) part to which the enzyme owes its specificity; its ability to act on a specific substrate.

By coenzyme is meant the part which accomplishes the action of the enzyme on the substrate. It is also called the "prosthetic" or "do something" part of the enzyme.

One of the big steps forward in the understanding of the behavior of certain vitamins was the discovery that they could and did form a structural part of certain coenzymes.

Thiamine, for example, as the cocarboxylase of the holoenzyme carboxylase (see Fig. 3).

Riboflavin as cytoflav, the coenzyme of Warburg's "Yellow Ferment."

Niacin in von Euler's Coenzymes I and II (Formerly called cozymases). *See Figs. 4 and 5.*

The term vitazyme in Schopfer's classification was coined to designate a vitamin known to form a part of a coenzyme. The group of enzymes in which vitamins form parts of the coenzyme fraction which have been most fully studied for their relation to metabolism are the so-called *respiratory enzymes.* From this and the behavior of these enzymes and coenzymes in cell oxidations has developed an explanation of the action of some of our well known vitamins.

CELLULAR OXIDATION

Early in the study of muscle behavior it was proven that the energy of the muscle was derived from the combustion or oxidation of glucose to carbon dioxide and water; that for each molecule of

68 calories of energy.

We sometimes express this fact the

Glucose + Oxygen → Car
$C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ C$

But this simple equation
molecule of glucose are ind

with 6 atoms of oxygen. The solution of this problem is one of the most brilliant chapters of chemical investigation and has revealed the role played in the process by certain vitamins.

To the chemist the term "oxidation" does not always mean the union of a substance with oxygen. Such combinations are one type of oxidation and oxides such as water, metallic and non-metallic oxides and the formation of organic acids from aldehydes are examples of this type.

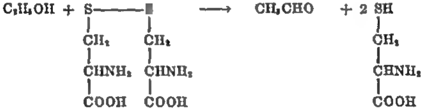
But there are other ways to accomplish the same effect, e.g.

(a) Ethanol + O → Acetaldehyde + Water

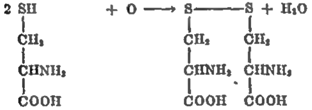
$$C_2H_5OH + O \rightarrow CH_3{\cdot}CHO + H_2O$$

In this operation the ethanol is oxidized to acetaldehyde and water. In this case the oxygen does not unite with the ethanol but does act on it to pull out two hydrogen atoms. This operation can be performed by other chemical compounds than oxygen with the same result; the same conversion of ethanol to acetaldehyde. For example:

(b) Ethanol + Cystine → Acetaldehyde + Cysteine



(c) 2 Cysteine mols. + O → Cystine + Water



In this example cystine is just as effective as oxygen in Equation (a) in converting ethanol to acetaldehyde and so long as oxygen is available cysteine can be converted back to the cystine form and the process continued indefinitely so long as there is any ethanol to lose hydrogens and oxygen to convert the cysteine back to cystine. But we now have a two step oxidation process. The first step is the removal of two hydrogens from the ethanol by a non-oxygen hydrogen acceptor (Cystine). The second step is the passing on of the hydrogen by the resultant cysteine to oxygen itself with the ultimate formation of water and release of energy.

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CELLULAR OXIDATION

Early in the study of muscle behavior it was proven that the energy of the muscle was derived from the combustion or oxidation of glucose to carbon dioxide and water; that for each molecule of water formed we get 68 calories of energy.

We sometimes express this fact by the following simple equation:

$$\text{Glucose} + \text{Oxygen} \rightarrow \text{Carbon dioxide} + \text{Water} + \text{Calories of Energy}$$
$$C_6H_{12}O_6 + 6\,O_2 \rightarrow 6\,CO_2 + 6\,H_2O + 408\ \text{Calories}$$

But this simple equation does not explain how the 12 hydrogens in each molecule of glucose are induced to leave the glucose molecule and unite

with 6 atoms of oxygen. The solution of this problem is one of the [illegible] brilliant chapters of chemical investigation and has revealed the [illegible] played in the process by certain vitamins.

To the chemist the term "oxidation" does not always mean the [illegible] a substance with oxygen. Such combinations are one type of [illegible] and oxides such as water, metallic and non-metallic oxides and the [illegible] tion of organic acids from aldehydes are examples of this type.

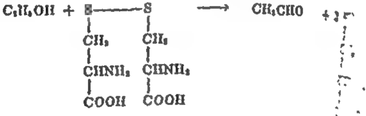
But there are other ways to accomplish the same effect, e.g.

(a) Ethanol + O → Acetaldehyde + Water

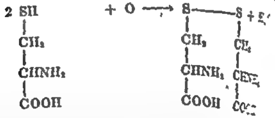
$C_2H_5OH + O \rightarrow CH_3{\cdot}CHO + H_2O$

In this operation the ethanol is oxidized to acetaldehyde [illegible] this case the oxygen does not unite with the ethanol but [illegible] to pull out two hydrogen atoms. This operation can be [illegible] other chemical compounds than oxygen with the same [illegible] conversion of ethanol to acetaldehyde. For example:

(b) Ethanol + Cystine → Acetaldehyde + [illegible]



(c) 2 Cysteine mols. + O → Cystine + [illegible]



In this example cystine is just as effective as oxygen [illegible] converting ethanol to acetaldehyde and so long as [illegible] cysteine can be converted back to the cystine form [illegible] tinued indefinitely so long as there is any ethanol [illegible] oxygen to convert the cysteine back to cystine. [illegible] step oxidation process. The first step is the removal [illegible] the ethanol by a non-oxygen hydrogen acceptor [illegible] step is the passing on of the hydrogen by the [illegible] itself with the ultimate formation of water and [illegible]

We will find that in tissue oxidations this use of non-oxygen hydrogen acceptors and a step by step transport of hydrogen from the initial metabolite such as sugar to its ultimate union with oxygen by use of such acceptors is quite the usual process, that hydrogen transport in the body takes place by steps, using a series of different hydrogen acceptors before ultimate union of the hydrogen with oxygen to form water. With that discovery we come still closer to the behavior of certain vitamins as hydrogen acceptors.

But there is still another way in which oxidation can take place. Change in valence is called oxidation when the change results in increased valence; for a metal e.g., the change of ferrous iron to ferric iron. What happens in this case? We know today that atoms contain a combination of positive and negative electrical charges; the positive charges called protons and the negative charges called electrons; that they can gain or lose electrons and thus become positively or negatively charged ions: e.g.

Ferrous iron less 1 electron becomes ferric iron

$$Fe^{++} - 1 \text{ electron} \rightarrow Fe^{+++} \text{ and}$$
$$Fe^{+++} + 1 \text{ electron} \rightarrow Fe^{++}$$

i.e., the change from ferrous to ferric iron (increase in valence or oxidation) means loss of an electron; the change from ferric to ferrous iron (reduction in valence or reduction) results from gain of an electron. Similarly an atom of hydrogen by loss of an electron becomes a positively charged hydrogen ion (H^+) and an atom of oxygen by gain of electrons becomes a negatively charged oxygen ion (O^{--}).

How could such transfer of electrons bring about the oxidation of a product such as ethanol to acetaldehyde with ultimate formation of water? Assume we have in a system ethanol (substrate); a hydrogen acceptor (cystine); a ferric iron ion (Fe^{+++}); and oxygen. These steps could then take place:

(d) Ethanol + cystine → acetaldehyde + cysteine as in Eq. (a)

The hydrogen is transferred from the substrate ethanol to the hydrogen acceptor cystine which is thus converted to cystine and the ethanol is oxidized to aldehyde. But now assume instead of handing their H atoms directly to an atom of oxygen these hydrogen atoms passed an electron to each ion of ferric iron. Let's assume this took place between 4 atoms of hydrogen from 2 molecules of cystine and four ferric ions. We would get this:

(e) $4\ Fe^{+++} + 4$ electrons from 4 H atoms $\rightarrow 4\ Fe^{++} + 4\ H^+$ ions

nd suppose further that those 4 ferrous ions instead of ... electrons, pass them right on to a molecule of oxygen (O_2) ... ct this:

(f) $$O_2 + 4 \text{ electrons from } 4\ Fe^{++} \rightarrow 4\ Fe^{+++} + 2\ O^{--} \text{ atoms}$$

Now we have our iron back to the ferric state ready to accept more ... and we have in solution 4 positively charged H^+ ions and 2 doubly ... y charged oxygen atoms. If now these meet, the O^{--} atoms, ... existing as free ions, combine with H^+ as they are formed to make ... cules of neutral water.

(g) $$4\ H^+ + 2\ O^{--} \rightarrow 2\ H_2O$$

We have exactly the same end result as in equations (b) and ... additional step in the oxidation process.

In tissue cells this last operation of electron transfer from ... atoms on a hydrogen acceptor to oxygen by way of a metal such ... actually accomplished by compounds called cytochromes (for ... see Fig. 21). So far as is known today, such cytochromes ... vitamins in their structure; they owe their part in step oxidation ... metal in the porphyrin ring

We can then, at this point, formulate these definitions of oxidation and reduction:

A product is oxidized when it unites with oxygen.
A product is oxidized when it loses hydrogen.
A product is oxidized when it loses electrons.
A product is reduced when it gains hydrogen.
A product is reduced when it gains electrons
An oxidizing agent is one that adds oxygen to a compound, or removes hydrogen from a compound, or removes electrons from a compound ...

RESPIRATORY ENZYMES

It is evident from the preceding discussion that to accomplish ... oxidation we must have hydrogen acceptors and donors ... acceptors and donors as well as oxygen itself. But are they ... You can expose glucose to the air and nothing happens. You ...

electrons on to the oxygen and the oxygen will not accept ... some additional stimulus.

It became necessary then to seek some catalytic agent ... would "activate" the hydrogens in the substrate and ...

the original habitat. It was also necessary to "activate" the oxygen to make it receptive of hydrogen atoms or electrons.

Such compounds were found in what are today called dehydrogenases and oxygenases; or collectively, respiratory enzymes.

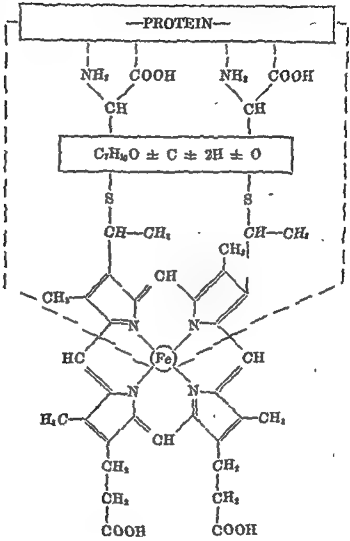


FIG. 21. CYTOCHROME "C"

Warburg's and Christian's "Yellow Enzyme" is such a compound and Theorell demonstrated it to be composed of a colloidal protein apoenzyme fraction linked to a phosphorylated vitamin, the vitamin B_2 or riboflavin (see Fig. 4). In this holoenzyme the phosphorylated riboflavin (also called cytoflav) is the prosthetic group or coenzyme part.

This particular enzyme is of special interest because it was the first to

be shown to owe its action in oxidation to the presence of a vitamin acting as a "hydrogen acceptor" and "hydrogen passer." The explanation of its behavior is as follows:

(1) The apoenzyme part, the protein fraction, gives it its specificity; that is, determines on what substrate it can act. We don't know just how this is accomplished. One explanation is that the protein is able to adsorb on its surface only particular substrates and thus bring them close enough to the prosthetic group for chemical reaction. Another explanation is that of Michaelis, who believes there is an intermediate compound formed between enzyme and substrate upon which the velocity of the action depends. At any rate, it is certain that the protein or apoenzyme fraction is what gives the holoenzyme specificity.

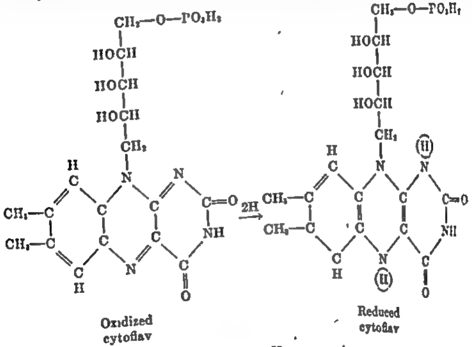


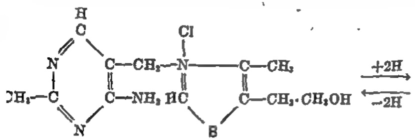
FIG. 22. CYTOFLAV AS A HYDROGEN ACCEPTOR

(2) The phosphorylated riboflavin or prosthetic group acts as a hydrogen acceptor which can receive from the substrate its hydrogens which the apo-enzyme ...

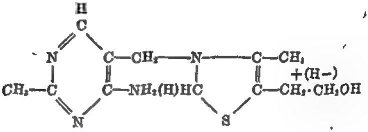
(a) The glucose gives up 2 hydrogen...
(b) The phosphorylated oxidized riboflavin fraction accepts those 2 hydrogens and is converted into the reduced form.

and noting what must be added to restore normal growth. Such studies not only established the fact that there were substances, hitherto unknown, necessary for growth but also that vitamin deficiencies could produce specific pathological and clinical responses. But to say that a vitamin is necessary for growth or for prevention of a specific disease such as scurvy, rickets, or pellagra does not explain *how* it acts or the relation of its activity to its chemical structure.

That is why the discovery of the relation of certain vitamins to respiratory enzymes and their action in metabolism is of such great significance. It is a step forward toward understanding of not only *what* vitamins do, but *how* some of them, at least, accomplish their effect.



Thiamine chloride



Reduced thiamine

FIG. 24. H TRANSPORT BY THIAMINE

The plant physiologists are today contributing further data on vitamin behavior. There is not space in this text for details of this type of research and its results, but it is worth while to illustrate its character. For example, it has been found possible to separate plant embryos from their cotyledons and endosperm materials in seeds and to grow them on synthetic culture media; paralleling the procedure in animal studies. By such a procedure it has been proved that they must be supplied with certain vitamins or growth ceases and to determine what particular vitamins are required by the embryos used in the test. It has also been found possible to follow similar procedure with parts of a plant b[illegible] ple, excised roots, and differentiate betw[illegible] requireme[illegible] and stems. Such studies, however, hav[illegible] ealed [illegible]

lved in growth stimulation but also the parts of the vitamin structure lved in certain instances.

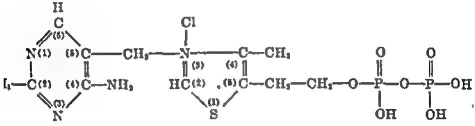
onner (1940), for example, has reported results which are tabulated in le VII. But Bonner also studied the structures in a vitamin compound

TABLE VII

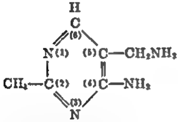
Vitamins Required by Plant Roots

(After Bonner)

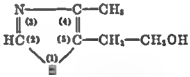
PLANT ROOTS	VITAMINS REQUIRED OR NOT REQUIRED		
Kinds	Thiamine	Niacin	Pyridoxine
. . . .	Yes	No	No
ish.	Yes	Yes	No
lfa	Yes	Yes	No
'er . .	Yes	Yes	No
on . .	Yes	Yes	No
'ot . .	Yes	No	Yes
ato.	Yes	Yes	Yes



(a) Cocarboxylase



(b) Pyrimidine part of thiamine chloride



(c) Thiazole part of thiamine

FIG. 25. SIGNIFICANT GROUPS IN THE COCARBOXYLASE MOLECULE

that were essential to its action. For example, in thiamine we have the thiazole and pyrimidine parts (shown in Fig. 25). Bonner proved that the "H" of the thiazole part in position 2 is of prime importance; that the CH_3 group in position 4 can be substituted without destroying activity; that

TABLE IX

*Recommended Dietary Allowances**

Food and Nutrition Board, National Research Council

	CALORIES	PROTEIN	CALCIUM	IRON	VITAMIN A***	THIAMINE (B_1)	RIBOFLAVIN	NIACIN (NICOTINIC ACID)	ASCORBIC ACID	VITAMIN D
		gms.	gms.	mgm	I.U.	mgm.	mgm	mgm.	mgm.**	I.U.
Man (70 kg.)										
Sedentary........	2500					1.5	2.2	15		
Moderately active..	3000	70	0.8	12	5000	1.8	2.7	18	75	†††
Very active........	4500					2.3	3.3	23		
Woman (56 kg.)										
Sedentary ..	2100					1.2	1.8	12		
Moderately active.	*2500*	*60*	*0.8*	*12*	*5000*	1.5	2.2	15	70	†††
Very active....	3000					1.8	2.7	18		
Pregnancy (latter half)	2500	85	1.5	15	6000	*1.8*	*2.5*	*18*	*100*	*400 to 800*
Lactation	*3000*	*100*	*2.0*	15	8000	2.3	3.0	23	150	400 to 800
Children up to 12 yr.										
Under 1 year† ...	100/kg.	3 to 4/kg.	1.0	*6*	1500	0.4	0.6	4	*30*	*400 to 800*
1 to 3 years†† .	1200	40	1.0	7	2000	0.6	0.9	6	35	†††
4 to 6 years. ...	1600	50	1.0	8	2500	0.8	1.2	8	50	
7 to 9 years ...	2000	60	1.0	10	3500	1.0	1.5	10	60	
10 to 12 years .	2500	70	1.2	12	4500	1.2	1.8	12	75	
Children over 12 yr.										
Girls,										
13 to 15 years	2800	80	1.3	15	5000	1.4	2.0	14	80	†††
16 to 20 years .	2400	75	1.0	15	5000	1.2	1.8	12	80	
Boys,										
13 to 15 years.	3200	85	1.4	15	5000	1.6	2.4	16	90	†††
16 to 20 years...	3800	100	1.4	15	6000	2.0	3.0	20	100	

* Tentative goal toward which to aim in planning practical dietaries; can be met by a good diet of natural foods. Such a diet will also provide other minerals and vit [illegible] are less well known.

** [illegible]

*** [illegible] ided chiefly *as the pro-vitamin carotene.*

† Needs of infants increase from month to month. The amounts given are for approximately 6-8 months. The amounts of protein and calcium needed are less if *derived from human milk*

†† Allowances are based on needs for the middle year in each group (as 2, 5, 8, etc.) and for moderate activity.

††† Vitamin D is undoubtedly necessary for older children and adults. When not available from sunshine, it should be provided probably up to the minimum amounts recommended for infants.

TABLE IX—*Continued*

Further Recommendations, Adopted 1942:

The requirement for *iodine* is small; probably about 0 002 to 0 004 milligram a day for each kilogram of body weight. This amounts to about 0 15 to 0 30 milligram daily for the adult. This need is easily met by the regular use of iodized salt, its use is especially important in adolescence and pregnancy.

The requirement for *copper* for adults is in the neighborhood of 1 0 to 2 0 milligrams a day. Infants and children require approximately 0 05 per kilogram of body weight The requirement for copper is approximately one-tenth of that for iron.

The requirement for *vitamin K* is usually satisfied by any good diet. Special consideration needs to be given to newborn infants. Physicians commonly give vitamin K either to the mother before delivery or to the infant immediately after birth.

TABLE X

The Basic Seven

Group I.	Green and yellow vegetables. One raw, one cooked, frozen or canned
Group II.	Oranges, tomatoes, grapefruit, or raw cabbage or salad greens
Group III.	Potatoes and other vegetables and fruits Raw, dried, cooked, frozen, or canned
Group IV.	Milk and milk products. Fluid, evaporated, dried milk, and cheese
Group V.	Meat, poultry, fish or eggs Or dried peas, beans, nuts or peanut butter
Group VI	Bread, flour, and cereals. Natural whole grain, enriched or restored
Group VII.	Butter or vitamin A fortified margarine
N.B.:	Serve daily one item from each group

CLINICAL DATA

Methods of determining accurately blood, urine and fecal content of certain vitamins are available today. As they are extended to larger and larger groups it is hoped that standards will emerge that will make possible routine clinical estimates of adequacy or normalcy similar to our blood and urinary tests for sugar in the diagnosis of diabetes mellitus. It is already rather widely accepted, for example, that a blood content of less than 0.5 mgm. per 100 cc. indicates inadequacy of vitamin C intake.

By giving large doses of vitamins and noting excretion in a given period of time, it is possible to determine whether the storage reservoirs of the body are saturated or depleted.

Special tests and apparatus are now available for detecting both acute and incipient avitaminosis. The biophotometer or adaptometer tests to detect incipient or acute vitamin A deficiency; eye examinations to reveal A and B_2 deficiency; the use of the x-ray to reveal D and C deficiencies in bone structures, are examples of progress in this direction. What has

TABLE XI

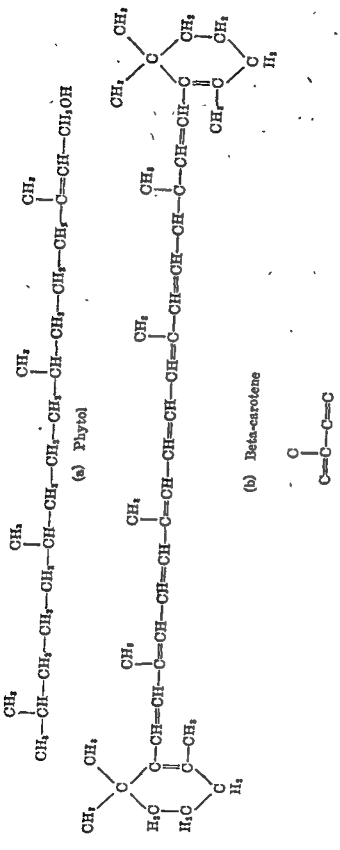
Special Tests for Vitamin Deficiency

A.	Biomicroscopy. Spectrometric blood
B_1.	Plasma content, blood pyruvic acid, electrocardiograms
B_2.	Biomicroscopy
Niacin.	*Tongue*
C.	Plasma C, capillary fragility, x-ray
D.	Serum Ca; P and phosphatase
K.	Prothrombin time (Quick)

DEFICIENCY SYMPTOMS	VITAMINS RELATED TO THE CONDITION									
	A	B_1	B_2	P-P	B_6	C	D	E	K	P
e Diseases										
Iild photophobia	X		X							
Iyctalopia (Night Blindness)	X		X							
Ietaplasia cornea and conjunctiva	X									
Kerosis and Bitot's Spots	X									
Iecrosis and ulceration corneal, lacrimal failure	X									
Pupil dilatation (mydriasis) and spot pigment on iris	X		X							
Vascularization of margin sclero-corneal junction			X							
Corneal inflammation (keratitis rosacea)			X							
Central ophthalmoplegia (paralysis of eye muscles)		X								
in Manifestations										
Dryness and roughness	X									
Increase in pigmentation	X		X							
Tendency to shark-like skin	X									
Acne form lesions	X									
Papular eruptions, hyperkeratosis hair follicles	X									
Symmetrical lesions resembling sunburn				X						
Atrophy of skin; scaling, cracking, bleeding				X						
Inflammation lips; fissures at corners			X							
Hemorrhage around hair follicles						X				
Petechial hemorrhage spots										X
Hemorrhagic manifestations						X			X	
Vaginitis	X			X						
euro muscular Manifestations										
Cramps in calf muscle		X								

TABLE XI—*Continued*

DEFICIENCY SYMPTOMS	VITAMINS RELATED TO THE CONDITION									
	A	B_1	B_2	P-P	B_6	C	D	E	K	P
Neuro-Muscular Manifestations —*Continued*										
Peripheral neuropathy: paresthesia or altered sensation, burning, tingling		X								
Polyneuritis, accompanying alcoholism, pregnancy (nerve inflammation)		X								
Sclerosis, amyotrophic lateral (over-growth of connective tissue in spinal cord)								X		
Muscular atrophy and dystrophy (faulty nourishment)								X		
Cardio-vascular Manifestations										
Tendency to hemorrhage									X	
Dyspnea (labored breathing), palpitation, rapid pulse, fatigue		X								
Sudden circulatory collapse		X								
Enlarged heart		X				X				
Edema, serous effusions (no congestive heart failure)		X								
Mucosal Manifestations										
Sore tongue, sore gums, sore mouth					X					
Glossitis (inflammation of tongue)			X							
Swelling, sponginess, bleeding of gums						X				
Cheilosis (cheilitis, perleche) fissures at mouth corners			X		X					
Bone Manifestations										
Periostitis, painful joints and bones						X				
Enlargements; rachitic rosary, Harrison groove, cranio tabies							X			
Contractions extremities, deformities spine and pelvis							X			
Reproduction Manifestations										
Threatened abortion								X		
Placental tearing								X		
Psychological Manifestations										
Irritability		X		X						
Nervousness . .			X	X			X			



(a) Phytol

(b) Beta-carotene

(c) Isoprene group

FIG. 26. RELATION OF PHYTOL AND BETA-CAROTENE

only if to the medium, a fragment of liver was added. The conclusion was that the liver fragment supplied the necessary carotenase. The evidence is obviously not conclusive since the carotenase enzyme has not been actually isolated from the liver and its action demonstrated in vitro.

FORMS OF VITAMIN A AS RELATED TO FUNCTION

It is now established that not only is vitamin A derivable from carotenoids by hydrolysis or oxidation but that the resulting vitamin may exist as an alcohol or as an ester and that there are differences in the biological behavior of these forms of A. There is also a difference between the absorption and utilization of carotenoids and that of the alcohol and ester forms of vitamin A.

TABLE XIII

Relative Potency of Vitamin A Forms

SOURCE OF A FED	TOTAL A FED	TOTAL A RECOVERED	RECOVERY
	U S P. Units		*Per cent*
U.S.P. Ref. Cod Liver Oil	273000	152000	55.7
Vitamin A caproate	292000	136000	46.5
Distilled A ester concentrate	288000	127400	44.2
Vitamin A stearate	300000	132800	44.3
Vitamin A alcohol	300000	118000	39.3
Beta-carotene	287000	27700	9.7

In the fish liver, vitamin A occurs as the ester and probably in that form in all fish liver oils. Gray, Hickman and Brown (1940) give the following percentage storage of various forms of A based on examination of rat livers after feeding types of A (see Table XIII).

These figures would indicate that the ester forms of A are better absorbed than the alcohol form and both forms definitely better than carotene.

Booher, Collison and Hewston (1939) using the Hecht and Schlaer adaptometer to measure physiological minima to prevent hemeralopia, reported carotene to be only 50 to 60 percent as effective as vitamin A in oil and Sherman (1941) uses these figures to suggest the human requirement of adults as 25–55 units of A or 43–103 units as carotene per kilo of body weight.

Bessey and Wolbach call attention to the fact that the amounts of vitamin A or carotene absorbed are affected:

(a) By the quantities administered
(b) Manner of administration
(c) Influence of other substances
(d) Degree of saturation of the body
(e) General physiological condition of the tract

and McCoord et al. (1934) state that amounts stored in liver are a better criterion of absorption than amounts in the circulating blood.

The relative solubility of the alcohol, ester and carotene in the intestinal tract naturally affect the rate of absorption. Carotene, for example, is more soluble in mineral oil than the two vitamin forms and hence less apt to be absorbed when taken with mineral oils. The absorption of fats is improved by bile salts and for that reason the vitamin esters are more readily absorbed when bile salts are present. Carotene cannot form esters and hence its absorption is less influenced by bile flow.

In dosage, it is probably justifiable to consider the relative values of these sources as best for those containing A esters; next best for those containing the free alcohol, and to definitely feed more units if the source is carotene.

METAPLASIA AND VISION

The specific effects of vitamin A deficiency will be discussed under "Avitaminosis A," (Chapter XVI). There are, however, in addition to the non-specific effect on growth, evidence that vitamin A supplies a substance necessary to the epithelial tissues and the formation of certain retinal pigments used in vision. The changes in tissue structure due to A deficiency are spoken of as metaplasia. *The chemical reactions through which carotene or vitamin A prevents this condition are still unknown. The chemical combinations necessary for retinal pigment formation are better understood and belong properly in this chapter.*

Carotenoids in Eyes

The first image forming eyes are found in arthropods and molluscs. The squid eye is a large double plano-convex lens with a retina composed of a single layer of cells containing a photosensitive pigment turned toward the light. Arthropod eyes are of the compound mosaic type, consisting of a number of units separated by pigments and each unit a complete dioptic apparatus.

The vertebrate eye is a third type. The retina is multilayered and the light receptor cells are of two types, rods and cones, which face away from the light.

The retinas of invertebrates contain high concentrations of vitamin A_1. But in them the A appears almost wholly concentrated in the eyes, little in other tissues, indicating that in these animals its relation to vision is its pre-eminent function. The squid retina contains 1–2 micrograms of A_1 and about three times that quantity of retinene, the active carotenoid. The quantity of A_1 is constant in all states of exposure to light and darkness with no evidence of participation in the visual process. The pigments

of the arthropods have been less extensively studied but it can be stated that the eyes of all invertebrates so far examined contain A_1, some of them in the form of retinene.

From the eyes of vertebrates two carotenoid pigments have been isolated—rhodopsin and porphyropsin. Rhodopsin is a rose-colored carotenoid protein with a maximal absorption band at 500 $\mu\mu$. In light it bleaches to orange or yellow products liberating in the process the carotenoid called retinene. The carotenoid in rhodopsin is vitamin A_1.

Porphyropsin is also a carotenoid protein but the vitamin combined with the protein is A_2, not A_1. It is distinctly purple in color and its maximal absorption band is at about 522 $\mu\mu$.

The behavior of the rhodopsin and porphyropsin systems are identical; the kind varies with the species. Their distribution is apparently as follows:

Marine fishes and land vertebrates—rhodopsin
Amphibia—rhodopsin or porphyropsin
Catadromous fishes—more rhodopsin than porphyropsin
Anadromous fishes—more porphyropsin than rhodopsin
Fresh water vertebrates—porphyropsin
Arthropods and Molluscs—rhodopsin and retinene 1.

The rhodopsin and porphyropsin proteins are found in the retinal rods. The chemical changes that take place when light impinges on the rods are indicated in Table XIV.

The maximal absorption spectra of these pigments and their relation to other carotenoids is shown in Table XV.

Put into words, the relation of these rod pigment changes may be stated as follows: the eye, like the camera, possesses a lens which focuses the image on the retina. The retina corresponds to the plate or film in the camera. We know that in the photographic plate or film the image is made visible because of a chemical change that has taken place in the silver emulsion coating when the light strikes it. We may compare the retinal pigments to the silver salts on the plate or film. The chemical changes induced by light on rhodopsin or porphyropsin provide the stimulus to the optic nerve which flashes the picture to the brain.

But in the camera, unless you remove the plate or move the film, you get only one picture. In the eye "reloading" is accomplished by regenerating the bleached rhodopsin or porphyropsin, i e. by regenerating retinene *1* or *2* to the original pigment. This requires a continuous and definite supply of vitamin A_1 or A_2 to the retinal layer, otherwise adaptation to dim light is slow, a condition described as night blindness or hemeralopia.

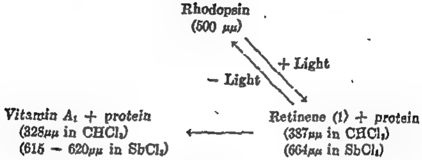
Retinene is a yellow pigment of a carotenoid nature. It is formed when light strikes the rhodopsin or porphyropsin. The action appears to be a

separation of the retinene and a protein since when vitamin A, retinene and a protein are combined rhodopsin is regenerated and the eye set to another picture.

TABLE XIV

Retinal Pigment Systems

A. *Rhodopsin System:*



B. *Porphyropsin System:*

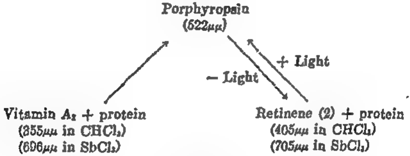


TABLE XV

Absorption Data

LIGHT	WAVE LENGTHS	CAROTENOIDS	MAX. ABSORPTION @
	$\mu\mu$		$\mu\mu$
Infra red	>700	Alpha-carotene	477
Red	700	Beta-carotene	485
Orange	600	Gamma-carotene	496
Yellow	580	Vitamin A_1 in $CHCl_3$	328
Green	500	Vitamin A_2 in $CHCl_3$	385
Blue	450	Rhodopsin	500
Indigo	420	Porphyropsin	522
Violet	400	Vitamin A_1 in $SbCl_3$	615–620
Ultra violet	<400	Vitamin A_2 in $SbCl_3$	696

Cone Pigment

Rhodopsin, or *rod pigment, is also called "visual purple."* The rods are concerned primarily with responses to dim light. The retinal cones are

concerned with color and bright light vision. According to Hecht (1939) they also contain a pigment, visual violet, which also requires vitamin A for its regeneration. The steps involved have not been clarified as in the rod pigment changes.

METHODS OF ASSAY FOR VITAMIN A AND CAROTENE

The method of establishing the unit potency of a source prescribed by the U. S. Pharmacopeia is a growth test using specially bred and pre-fed white rats. The growth effect of the source is matched against an amount of a reference standard whose potency is known.

Because of the fact that vitamin A and carotene produce a characteristic blue color with antimony chloride ($SbCl_3$) and because the intensity of such a color can be used to indicate concentration of the vitamin, colorimetric tests using the Lovibond tintometer or an electrophotometer have been devised suitable for estimating concentration of extracted vitamin A or carotene.

A third method depends on measurement of the absorption of certain wave lengths by solutions of vitamin A in suitable solvents and using a spectrophotometer.

And recently, Popper has been able to demonstrate vitamin A in tissues because of its ability to fluoresce when acted upon by ultraviolet light. See Plate V, page 154 By use of one or more of these various methods it has been possible to examine blood, urine, tissues, etc., and quantitatively determine their vitamin A or carotene content.

For further details on assay methods, see Part III.

MEASURING HUMAN REQUIREMENTS

Using such assay methods we now have available certain data bearing on the normal blood content of vitamin A and carotene. Both forms are normally present in circulating blood. Such data contrasted with individual blood samples have been used to determine normalcy of intake and to diagnose deficiency.

Another method of establishing human requirements has been the use of the dark adaptation measurements and the determination of least amounts necessary to restore vision to normal. The value of such tests and their significance in establishing human requirements will be reviewed in the chapter on Avitaminosis A.

CHAPTER VI

THE NATURE AND FUNCTION OF THIAMINE (VITAMIN B_1)

In 1914 Funk, by contrasting diets containing a high percentage of carbohydrate with those high in fat or protein, showed that the onset of polyneuritic symptoms came much earlier on the high carbohydrate diets. This was the first suggestion that vitamin B_1 needs were related to fuel supply and to carbohydrate fuel in particular.

Later Cowgill confirmed the relation of the B_1 requirement to calorie intake by a series of studies with different species of animals. He found that given the weight of the animal and the calorie intake one could predict, by use of a formula, the minimum amount of B_1 necessary to prevent visible symptoms of polyneuritis. At the time, Cowgill did not have pure thiamine to work with but used a concentrate and expressed his vitamin B needs in milligram equivalents of this concentrate (see Table III). Later it was shown that Cowgill's milligram equivalent was equal to 0.00015 milligrams of thiamine. Cowgill's original formula was given as follows:

$$\frac{\text{Vitamin B Needs in Milligram Equivalents}}{\text{Calorie Intake}} K_s \times \text{Weight in Grams}$$

In this formula Ks is a constant for a given species. He determined these Ks values for the rat, the mouse, the pigeon, the dog and for man. The values determined are given in Table XVI.

Cowgill's milligram equivalent was later shown to be equal to 0.05 of an International unit of B_1 and since that unit is equal to 0.003 mgm. of thiamine, it is possible to rewrite this formula as follows:

(a) B_1 need in Int. units = 0.00142 × Wt. in Kilograms × Calories
(b) B_1 need in Int units = 0.00071 × Wt in Pounds × Calories
(c) B_1 need in microgm. = .00213 × Wt. in Pounds × Calories

On the basis of this formula, Jolliffe has worked out a prediction tabulation (Table XVII).

See also, Figure 27, after Cowgill, a chart from which the vitamin/calorie ratio necessary to prevent polyneuritis is given for any given weight. (N.B. In Jolliffe's vitamin/calorie ratio, vitamin is in International units of B_1).

From these figures and plots of the adequate vitamin/calorie ratio necessary to prevent polyneuritis, it would appear that the source of the calories

was immaterial. Williams and Spies have presented considerable data to support the view that only non-fat calories should enter into the prediction B_1/caloric ratio. To check this point Jolliffe recalculated 100 of Cowgill's dietaries associated and unassociated with beriberi and determined the following ratios: thiamine/calorie; thiamine/carbohydrate calories; thi-

TABLE XVI

Conversion Species Constants

K_s for the mouse	= 0.15
K_s for the rat	= 0.0099
K_s for the pigeon	= 0 0037
K_s for the dog	= 0.000076
K_s for man	= 0 0000284

TABLE XVII

(After Jolliffe)

B_1/Calorie Ratios for Adequacy

BODY WEIGHT		B_1/CALORIE RATIO	INT UNITS B_1 REQUIRED WHEN DIET SUPPLIES FOLLOWING CALORIES:				
			1500	2000	2500	3000	3500
kgm.	*lbs*						
40	88	1 20	90	120	150	180	210
45	99	1 33	100	133	166	200	233
50	110	1.47	110	147	184	220	257
55	121	1 62	121	162	202	243	283
60	132	1.77	134	178	222	266	311
65	143	1 91	143	191	239	286	333
70	154	2.05	154	205	256	307	359
75	165	2.22	166	222	277	333	390
80	176	2 40	180	240	300	360	420
85	187	2 53	190	253	316	279	443
90	198	2 65	200	266	332	398	464
95	209	2 80	210	280	350	420	490
100	220	2 95	221	295	369	442	516
105	231	3.10	232	310	387	465	542
110	242	3 25	244	325	400	487	569

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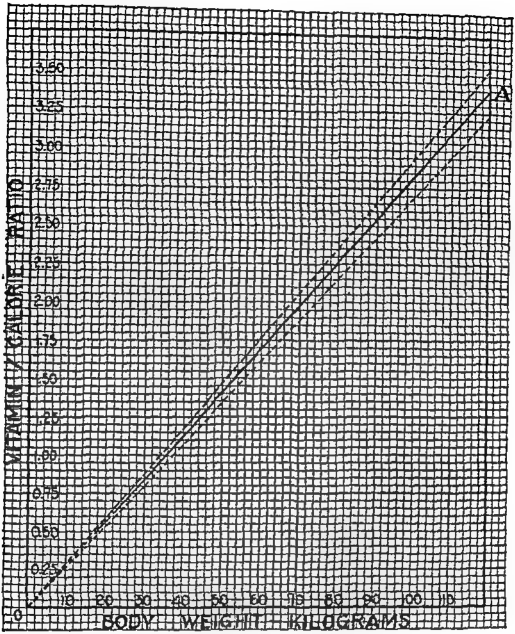

FIG 27. Cowgill's prediction chart for estimating the vitamin B adequacy of any given diet. "The plot indicated by line *OA* represents the probable minimum vitamin B(B_1) requirement referred to body weight The area between the dotted lines represents a zone of uncertainty. *If* the Vitamin/Calorie value of the diet for a given body weight falls definitely above line *OA*, the ration is deemed adequate with respect to vitamin B; if the plot proves to be appreciably below the line, the vitamin requirement is not satisfied by this diet and beriberi should occur provided the period of subsistence on this ration is sufficiently extended; if the plot is close to line *OA* or between the dotted lines, the diet may be considered as 'borderline' in character." (Reprinted by permission of the author)

gill formula. Williams and Spies incline to the view that the thiamine unit/calorie ratio is a better index and to a large degree independent of weight. They suggest the ratios shown in Table XIX as borderline values regardless of the individual weights. To their thinking, a B_1 unit/calorie ratio below 1.7 indicates danger of beriberi and above 2.29 safety from beriberi.

These studies of the direct relation between calorie intake and thiamine requirement suggested its action in energy metabolism and in the metabolism of carbohydrates in particular. Progress toward the solution of this action was greatly advanced by study of the composition of brains of B_1 deficient and B_1 normal pigeons in Peters' laboratory at Oxford; by Williams' determination of the chemical structure of thiamine, and by the discovery of cocarboxylase.

TABLE XVIII

(After Jolliffe)

Comparisons of B_1/Calorie Ratios

PREDICTION INDEX	NUMBER OF DIETARIES	IN ERROR	ERROR
			per cent
Thiamine/calorie ratio	85	4	4.7
Thiamine/carbohydrate calorie ratio	62	[illegible]	4.8
Thiamine/non-fat calorie ratio	83	3	3.6
B_1/calorie ratio (Cowgill)	65	1	1·5

TABLE XIX

Williams' and Spies' Ratios

Thiamine/calorie ratio	0.230–0.279
Thiamine/calorie ratio	1.21 –2.51
Thiamine/non-fat calorie ratio	0.251–0.300
Vitamin B_1 Units/calorie ratio	1.7 –2.29

COCARBOXYLASE

In 1911 Neuberg and Karczag reported the presence in yeast of carboxylase, an enzyme that catalyzed the decarboxylation of pyruvic acid to acetaldehyde and carbon dioxide. The crude enzyme also attacked other alpha-keto acids. Auhagen (1932) showed that for this action, the carboxylase required a thermostable coenzyme which could be removed from yeast by washing with alkaline phosphate, and in 1937 Lohmann and Schuster isolated this cocarboxylase from yeast as the crystalline hydrochloride

little or no synthesis in the absence of oxygen. Iodoacetic acid is strongly inhibitory. The optimum reaction for synthesis is about pH 8.5.

THIAMINE IN PLANTS

Thiamine is found in almost all groups of plants and in higher plants in all organs: roots, stems, leaves, fruits, flowers. By using the Phycomyces test, examination of leaves of 134 species of plants showed them to contain *one or more of the following: thiamine, its two components, pyrimidine* and thiazole, or a substance closely related to thiamine.

One of the results of this plant study has been to show that certain substitution products of thiamine exhibit some activity if used in relatively high dosage. Schultz, for example, using an animal test, has reported that of 39 homologues of thiamine, 22 were found with definite activity and there is definite evidence that plants contain substances related to but not identical with thiamine that have similar activity. A thiamine in which *the CH_3 group in position 2 of the pyrimidine part (see Fig. 25) is replaced* by C_2H_5 had greater activity than thiamine by the Phycomyces test.

Plants, too, are able to synthesize thiamine from its precursors and also to make thiazole. Bonner and Buchman, for example, by supplying thioformamide, chloracetopropyl alcohol, and pyridine to pear roots got as good growth as with thiamine. The first two products it converts to thiazole and then is able to combine the pyrimidine with the thiazole to make thiamine.

CHAPTER VII

THE NATURE AND FUNCTION OF RIBOFLAVIN (VITAMIN B_2 OR G)

As stated in Chapter II, riboflavin was first believed to be the heat stable part of what was then called "water soluble B." At that time it had been shown that aqueous extracts of sources of water soluble B contained at least two vitamins; one curative of beriberi and the other, more heat stable, necessary for growth and in some way concerned in the prevention of a dermatitis.

This first led to the belief that it was an antipellagric factor and early studies concentrated on the elucidation of this claim.

In that period the two vitamins received various designations. Goldberger called it the pellagra-preventive or P-P vitamin and the heat labile factor, the antineuritic vitamin. At that time, it was also called vitamin G and the heat labile factor retained the vitamin B designation. English investigators suggested B_1 and B_2 for the two factors and that proved to be a better choice as it soon developed that water extracts contained more than *two* vitamins. With its chemical identification the chemically significant name "riboflavin" became the preferred designation, just as thiamine is now preferred for the B_1 or antineuritic factor.

The dermatitis resulting from riboflavin deficiency proved, however, to be quite different from that characteristic of human pellagra or its analogue in dogs, blacktongue. This fact was brought out in several laboratories (Gurin and Eddy; Akroyd; Sure, Smith and Kik; Koehn and Elvehjem). The differentiation between the two types of dermatitis and the characteristics of the riboflavin deficiency type were elucidated by Hogan and is discussed in Chapter XVIII.

Sherman and Bourquin developed a rat growth test which not only proved the importance of riboflavin as a growth factor but, for a time, provided a method of statement of source potency (see Table III). Riboflavin content is still sometimes expressed in Sherman-Bourquin units but more generally today in actual weight, milligrams or micrograms.

RIBOFLAVIN AS A RESPIRATORY ENZYME COMPONENT

With the discovery (reviewed in Chapter II) that phosphorylated riboflavin was the prosthetic group in Warburg and Christian's Yellow Ferment, riboflavin's function in nutrition assumed special importance and concen-

little or no synthesis in the absence of oxygen. Iodoacetic acid is strongly inhibitory. The optimum reaction for synthesis is about pH 8.5.

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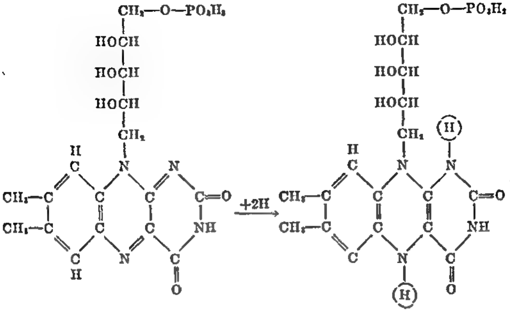
trated attention to its method of action in the hydrogen transport system essential to cellular and tissue oxidations. Phosphorylated riboflavin was isolated by Banga and Szent-Gyorgyi from pig heart in 1932, shown to act as a coenzyme, and called by them, "cytoflav" (see Fig. 22). It was isolated in its crystalline form by P. György in collaboration with Kuhn and Wagner-Jauregg in 1933.

In the discussion of Cellular Oxidation in Chapter III, it was explained that enzymes which bring about the removal of hydrogen from a compound are called dehydrogenases. Warburg's yellow enzyme is such a dehydrogenase. Since the structure of that enzyme was elucidated by Theorell (see Fig. 4) several other flavoprotein enzymes have been found (see Table VI). In all of them cytoflav or phosphorylated riboflavin provides the hydrogen acceptor. The actual prosthetic groups of these flavoprotein enzymes are either alloxazine mononucleotide or alloxazine adenine dinucleotide (see Fig. 28). All are characterized by reaction with oxygen—some to a limited extent—when in the reduced form. In the oxidized state, flavoproteins, as a group, can be reduced by a variety of substrates; e.g. by Coenzymes I and II, by the d-amino acids, by xanthine and other purines, and by some of the aldehydes. But each particular flavoprotein is specific toward one or one class of substrate (see Table VI, Chapter II).

The chain of hydrogen acceptors involved in the transformation of carbohydrate to CO_2 and water has been fairly well worked out. A place in this series has been found for Coenzymes I and II, for the electron acceptors and transporters known as cytochromes a, b, and c and for cocarboxylase. Somewhere in this series one or more flavoproteins are believed to function. As we understand the process today actual passing of hydrogen or electrons requires at each step some activator to make the hydrogens or electrons move to the next acceptor. If, for example, we start with a substrate like glucose, a dehydrogenase with a coenzyme is necessary to make the hydrogens leave the glucose and attach themselves to the coenzyme. But then it is necessary to have another activator to make them leave the coenzyme and pass to the next hydrogen acceptor. The chemist's problem has been to isolate and prove the existence of all the activators and hydrogen acceptors that are actually present in a given tissue oxidation system and the sequence of their action. Starting at the other end of the system there must also be oxygenases to make oxygen accept hydrogen and electrons. Ball has stated the following as the probable sequence:

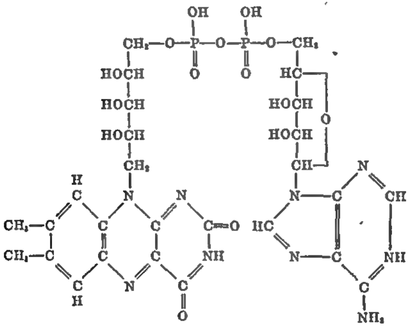
(a) A specific substrate under influence of a dehydrogenase protein passes hydrogen and/or electrons to an acceptor which is probably one of the pyridine nucleotides (Coenzyme I or II, see Fig. 5 and 23). As a result the pyridine acceptor is reduced, now has added hydrogen or electrons or both.



(a) Riboflavin-5′-phosphoric acid

Oxidized form

Reduced form



(b) Alloxazine Adenine dinucleotide

FIG. 28. ALLOXAZINE NUCLEOTIDES TRANSPORT OF H

(b) *This reduced pyridine now becomes a new substrate.* It, in turn, mus[t be] oxidized to make it pass on these hydrogens. There is evidence that [this] *oxidation is accomplished by flavo-proteins and the hydrogens attach th[em]selves to the flavin part of the flavo-protein.*

(c) The flavo-proteins with the reduced flavin part must now be oxidized to m[ake] them give up their hydrogen and go back to the oxidized state. H[ow?] For some time this problem remained unsolved. It was believed tha[t in] some way, *the reduced flavin product passed its electrons on to the cy[to]chromes (to cytochrome C) thus reducing the metallic cation in the cy[to]chrome nucleus and putting hydrogen ions into solution. Also that cy[to]chrome must be reduced to permit its acceptance of these electrons.* [The] discovery of cytochrome reductase by Hogness and Haas, which is a fla[vo-]protein, and that it reacts with cytochrome C appears to bridge the [gap] between the pyridine nucleotides and the cytochromes and confirms [the] view that the flavo-protein is the compound that gets electrons from [the] pyridine nucleotides to, at least, cytochrome C.

The whole chain then today appears to be capable of summary as follo[ws:]

1. Substrate	H
2. *Pyridine nucleotides*	|
3. *Flavoproteins*	|
4. Cytochromes	|
5. Oxygen	H_2O

If the substrate is pyruvic acid, it must be in some way first decarbox[yl]ated to start the steps from 2 to 5 and that, as we have seen, requi[res] cocarboxylase. *This scheme of oxidation then provides a specific pla[ce] and function for riboflavin.* It is an essential component of the flav[o-]proteins and hence essential to cellular respiration and to the comple[te] metabolism of carbohydrates. Like *thiamine, whose function we ha[ve] already discussed*, and niacin, which we will consider in the followi[ng] chapter, riboflavin becomes important in all diets as part of the machine[ry] the body uses *to get energy out of fuel food* such as carbohydrates.

RIBOFLAVIN IN PLANTS

Like *the carotenes, the yellow pigments* now classed as flavins have lon[g] been known and their presence in plants as well as in milk, liver, etc., note[d]. The fact that they are also found in bacteria as well as in *higher plant[s]* would suggest that chlorophyll is not necessary for their synthesis by th[e] plant. In fact little is known of the conditions required for the synthesi[s] of riboflavin, niacin or pyridoxine in plants. According to Lavollay an[d] Labosay, a deficiency of magnesium in the medium favors the formatio[n] of riboflavin in *Aspergillus niger*.

The first proof that riboflavin is required by certain plants for growt[h] resulted from the study of lactic acid bacteria. Snell and Strong hav[e] shown it an essential to the growth of eleven species of *lactic acid bacteria*.

A few species have been shown able to synthesize the vitamin. Little is known of its action in higher plants but its ability to act in hydrogen transport would suggest its value in any organism when oxidation processes are necessary to life. There is some evidence that riboflavin may be present in soil and that certain roots supply riboflavin by taking it from the soil.

OTHER FUNCTIONS OF RIBOFLAVIN

Riboflavin is an essential growth factor for animals. According to Snell (1939), to be fully active on animals the vitamin must have two methyl groups in the sixth and seventh positions. These may be replaced by tri and tetra methylene rings without loss of activity (cyclopropane and cyclobutane respectively). One of the methyl groups can be replaced by an ethyl group but substitution of a methyl group in the fifth or eighth position destroys the vitamin activity. The NH group in the third position must be free. The sugar group must have the alcoholic structure. Other chemical properties of riboflavin are reviewed in Chapter II.

The relation of riboflavin to prevention of disease is discussed in Chapter XVIII. The daily adult human requirement is now considered to be about 2.7 mgm. The therapeutic dose has been estimated at from 5 to 15 mgm. According to Kuhn it is not toxic in amounts as high as 20 grams.

There has been a suggestion that riboflavin may be a part or related to the extrinsic factor concerned in prevention of pernicious anemia. This view lacks confirmation to date.

(b) This reduced pyridine now becomes a new substrate. It, in turn, must be oxidized to make it pass on these hydrogens. There is evidence that this oxidation is accomplished by flavo-proteins and the hydrogens attach themselves to the flavin part of the flavo-protein.

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4. Cytochromes
5. Oxygen — H_2O

If the substrate is pyruvic acid, it must be in some way first decarboxylated to start the steps from 2 to 5 and that, as we have seen, requires cocarboxylase. This scheme of oxidation then provides a specific place *and function for riboflavin. It is an essential component of the* flavoproteins and hence essential to cellular respiration and to the complete metabolism of carbohydrates. Like thiamine, whose function we have already discussed, and niacin, which we will consider in the following chapter, riboflavin becomes important in all diets as part of the machinery the body uses to get energy out of fuel food such as carbohydrates.

RIBOFLAVIN IN PLANTS

Like the carotenes, the yellow pigments now classed as flavins have long been known and their presence in plants as well as in milk, liver, etc., noted. The fact that they are also found in bacteria as well as in higher plants would suggest that chlorophyll is not necessary for their synthesis by the plant. In fact little is known of the conditions required for the synthesis of riboflavin, niacin or pyridoxine in plants. According to Lavollay and Labosay, a deficiency of magnesium in the medium favors the formation of riboflavin in *Aspergillus niger*.

The first proof that riboflavin is required by certain plants for growth resulted from the study of lactic acid bacteria. Snell and Strong have shown it an essential to the growth of eleven species of lactic acid bacteria.

A few species have been shown able to synthesize the vitamin. Little is known of its action in higher plants but its ability to act in hydrogen transport would suggest its value in any organism when oxidation processes are necessary to life. There is some evidence that riboflavin may be present in soil and that certain roots supply riboflavin by taking it from the soil.

OTHER FUNCTIONS OF RIBOFLAVIN

Riboflavin is an essential growth factor for animals. According to Snell (1939), to be fully active on animals the vitamin must have two methyl groups in the sixth and seventh positions. These may be replaced by tri and tetra methylene rings without loss of activity (cyclopropane and cyclobutane respectively). One of the methyl groups can be replaced by an ethyl group but substitution of a methyl group in the fifth or eighth position destroys the vitamin activity. The NH group in the third position must be free. The sugar group must have the alcoholic structure. Other chemical properties of riboflavin are reviewed in Chapter II.

The relation of riboflavin to prevention of disease is discussed in Chapter XVIII. The daily adult human requirement is now considered to be about 2.7 mgm. The therapeutic dose has been estimated at from 5 to 15 mgm. According to Kuhn it is not toxic in amounts as high as 20 grams.

There has been a suggestion that riboflavin may be a part or related to the extrinsic factor concerned in prevention of pernicious anemia. This view lacks confirmation to date.

TABLE XX

(After Elvehjem, 1940)

Active and Inactive Derivatives of Niacin

ACTIVE	INACTIVE
Nicotinic acid	Pyridine
Nicotinic acid amide	Picolinic acid
Ethyl nicotinate	Isonicotinic acid
Nicotinic acid N methyl amide	Nipecotic acid
Nicotinic acid N diethyl amide	6-Methyl *nicotinic acid*
Beta-picoline	Trigonelline
Nicotinuric acid	1-Methyl-nicotinic acid amide hydrochloride
	Quinolinic acid
	Beta aminopyridine

TABLE XXI

Properties of the Coenzymes

Property	Coenzyme I	Coenzyme II
Empirical formula	$C_{21}H_{27}O_{14}N_7P_2$	$C_{21}H_{28}O_{17}N_7P_3$
Molecular weight	663	743
Structural units	1 mol. niacinamide 1 mol. adenine 2 mols. pentose 2 mols. phosphoric acid	1 mol. niacinamide 1 mol. adenine 2 mols. pentose 3 mols. phosphoric acid
STABILITY:		
Oxidized form In 0.1 N HCl @ 100° C.	50% destroyed after 8 minutes 50% destroyed after 17 minutes @ 20°C.	50% destroyed after 7 3 minutes 50% destroyed after 12 minutes @ 23°C.
Reduced form In 0.1 N HCl @ 20°C.	Activity disappears immediately	Activity disappears immediately
In 0.1 N NaOH @ 100 C.	Slight decrease in activity after 10 min.	
In 0.1 N NaOH @ 20°C.	*Stable*	Stable
Oxidized form Maximum @ 260 mu mu	$E = 3.8 \times 10^7 \left(\frac{cm.^2}{mol.}\right)$	$E = 3.5 \times 10^7 \left(\frac{cm.^2}{mol.}\right)$
Reduced form Maximum @ 260 mu mu	$E = 3.3 \times 10^7 \left(\frac{cm.^2}{mol.}\right)$	$E = 3 1 \times 10^7 \left(\frac{cm.^2}{mol.}\right)$
@ 340 mu mu	$E = 1.1 \times 10^7 \left(\frac{cm.^2}{mol.}\right)$	$E = 1 0 \times 10^7 \left(\frac{cm.^2}{mol.}\right)$

NIACIN IN PLANTS

According to Schopfer, niacin is a true growth factor for certain plants. It is required by several bacteria. It is not known to be required by any fungus, but it is required by the higher green plants. According to Landy, basing his observations on the effect of niacin on staphylococcus aureus, the niacinamide has ten times the activity of niacin. The N-ethyl amide and the sodium and ammonium salts of niacin are 1/5th as active as niacin. Trigonelline is inactive for staphylococcus.

Karrer and Benz state that all pyridines are able to undergo a reversible hydrogenation by virtue of their pentavalent atom of nitrogen and, in this respect, they are similar to the thiazole group of thiamine.

CHAPTER IX

THE NATURE AND FUNCTION OF PYRIDOXINE (VITAMIN B_6)

Pyridoxine, or vitamin B_6, was the third vitamin to be identified in water-soluble B complex. Its discovery and chemical identification ceded that of niacin. Chick and Copping were the first to produce evid of its existence and at the time designated it as factor "Y." It was re covered independently by Paul György. György differentiated the der titis produced by its deficiency from that due to B_2 and called the condi "acrodynia." The name "adermin" was first suggested but today

TABLE XXII

Relation of Thiamine and Pyridoxine to Plant Stimulation

	DRY WT OF CRO
	Milligrams
Control	0.4
5 gamma thiamine	3.4
5 gamma thiamine plus 1 gamma pyridoxine	16.1
1 gamma pyridoxine	1.8

accepted term is *pyridoxine.* Ohdake actually isolated the vitamin 1931 but its identification as a vitamin was at that time not yet clarifi Birch and György reported many of the chemical properties of vitamin and announcement of its crystallization was reported by Lepkovsky 1938. Keresztesy and his associates also announced the crystallizati of B_6 and only a little later, György reported its isolation; confirmed Kuhn and coworkers and by Ichiba and Michi.

In 1939 Harris and Folkers of Merck Laboratories and Kuhn and a sociates showed that vitamin B_6 was a pyridine derivative (see Fig. (A year later synthetic vitamin B_6 became available.

The presence of the pyridine ring would suggest that it might provic like niacin, material for the formation of coenzymes but we have no dire evidence of such action.

PYRIDOXINE IN PLANTS

Apparently most microorganisms synthesize pyridoxine and some r quire it for growth. It has also been shown that the excised roots of tomat at least of some varieties, require pyridoxine. Robbins and Bartle

Schmidt found that when pyridoxine was added to thiamine or thiazole, it increased the activity of these substances on tomato roots (see Table XXII). They state that pyridoxine exhibits little activity when used alone. It acts in a combination based on thiamine. According to Bonner, carrot, tomato, datura, and sun flower roots require pyridoxine.

Moller in 1938 identified the thermostable factor necessary for the growth of a bacterium as pyridoxine. He also reported that certain yeasts require pyridoxine. Moller also (1940) has reported the specificity of the action of pyridoxine on lactic acid bacteria.

GENERAL FUNCTIONS OF PYRIDOXINE

In the rat it prevents the specific dermatitis of acrodynia. Its deficiency has been reported to produce a microcytic, hypochromic anemia. It appears to be essential for hemoglobin formation but just how is unknown.

Birch et al. were the first to call attention to the fact that acrodynia produced on low fat diets could be more readily cured with pyridoxine if fat was also fed. They termed this phenomenon the sparing action of fat on pyridoxine. Birch advanced the idea that essential fatty acids are necessary for the utilization of pyridoxine and conversely, that pyridoxine was required for the utilization of the essential fatty acids.

CHAPTER X

THE NATURE AND FUNCTION OF THE "BIOS" NUTRILITES

In 1901 Wildier in Ide's laboratory in Louvain, Belgium, postulated the existence of a substance or substances needed in addition to a sugar-salt culture medium for the growth of yeast. He called these substances collectively "bios." Pasteur had claimed that yeasts would grow in a synthetic medium containing sugar, ammonium tartrate, and yeast ash minerals. Wildier maintained that the wort needed in addition, a minute amount of the organic matter he called "bios."

In 1919 R. J. Williams working in the F. C. Koch laboratory in Chicago advanced the view that bios and water-soluble B were identical. This contention initiated the study of the nature of bios in this country. In 1928 Williams suggested the name "nutrilites" for factors with bios activity for the following reasons:

> "The term vitamin carries unfortunate etymological implications and its use should not be unduly extended. The term *nutrilite* on the other hand, is unobjectionable from this standpoint. Since it implies only an *importance in nutrition* and *nothing as to the chemical nature* of the agent."

Fulmer, Duecker and Nelson (1923) showed that bios was not a single fact on but rather a complex of factors differing in their properties. This led to the postulation of bios I and bios II of Miller. Eastcott (1928) isolated bios I from tea leaves and proved it to be inositol. The bios II was a concentrate, not a single substance, but she got optimum growth by combining bios I and II. Bios II was then shown to consist of at least two substances, one not adsorbed on charcoal and one adsorbed on charcoal. In Lash Miller's nomenclature these were called bios IIa. and IIb. respectively. Kogl called Miller's bios IIb., bios II, and his bios IIa., bios III.

In 1933 R. J. Williams and associates postulated the existence of a nutrilite active on yeast to which was given the name of pantothenic acid. This product is undoubtedly one of the fractions associated with bios IIa. However, the position of pantothenic acid in the classification of bios I, IIa and IIb is still uncertain. Furthermore, it seems unnecessary to continue these classifications as bios fractions, since today biotin, inositol, pantothenic acid and folic acid, in addition to pyridoxine and thiamine, have been definitely characterized chemically and all are growth factors for yeast and for certain other organisms.

The nature and functions of thiamine, riboflavin, and pyridoxine have

already been outlined. The nature and functions of these other nutrilites follows.

INOSITOL

As stated above, inositol was first demonstrated by Eastcott in 1928 in Lash Miller's laboratory. She obtained the product from tea. This product has specific bios activity but becomes a limiting factor only when other nutrilities are present. It occurs free and in the form of phytic acid (see Fig. 9).

In 1940 Woolley showed that inositol was preventive of alopecia in mice. One form of denudation in rats was the loss of hair around the eyes and a swelling. This condition has been described as spectacled eye. Paveck and Baum report that inositol proved to be a specific cure for this condition.

Little is known as to the human requirement for inositol. Gavin and McHenry have reported that fatty livers produced by cholesterol feeding were prevented by inositol.

PANTOTHENIC ACID (PANTOTHEN)

In 1936 Lepkovsky, Jukes and Krause, after separation of thiamine and riboflavin from a water extract of rice polishings, demonstrated in the residuum two activating fractions which they called Filtrate Factors I and II. Factor I turned out to be the antiacrodynia factor that is now known as vitamin B_6 or pyridoxine. The other Filtrate Factor proved corrective of a dermatitis peculiar to chicks.

R. J. Williams in 1939 reported the recovery from extracts used to stimulate yeast growth of a compound whose calcium salt appeared to have the formula $(C_8H_{14}H_2O_5)_2Ca$. He found this substance not only stimulative to the growth of yeast but apparently essential for growth of all living cells. He also found it universally distributed in plant and animal tissues and it was for that reason that he gave it the name "Pantothenic Acid."

Williams' pantothenic acid salt cured chick dermatitis which Lepkovsky et al. reported to respond to their filtrate factor, thus indicating that at least that part of the filtrate factor corrective of the dermatitis was identical with Williams' pantothenic acid. Science Service reported R. J. Williams as saying:

> "Since its discovery pantothenic acid has been found to be not only present in widely different tissues and organisms but to function as a potent physiological substance stimulating the growth of yeasts, molds, lactic acid bacteria, diphtheria bacillus [illegible]
>
> [illegible]
>
> first one linking it up definitely as a 'growth-promoting' substance for higher ani-

chose "H" because it is the first letter in the German word for skin—"haut"—and he characterized the skin condition as of the seborrheid desquamative type. György also showed that the H in liver and yeast must be in a combined form because it was released only by hydrolysis of liver and autolysis of yeast; that the product when once freed was readily dialyzable and hence of small molecular weight; that it was acidic in character with isoelectric point between 3 and 3.5.

In 1928 he combined forces with duVigneaud. It developed that György's vitamin H was identical with Kögl's biotin and the structure of biotin was worked out by György and du Vigneaud et al. (see Fig. 10).

In 1940, Williams and coworkers produced evidence that the substance that caused egg white injury was united with biotin in a fairly stable combination but decomposed by hydrolysis. In 1941, Williams and György et al. tested the hypothesis that the injury was due to blocking the activity of biotin by its union with this protein factor in the raw egg white. Williams named this factor "avidin" because of its avidity for biotin. He obtained it in crystalline form and proved it to be a protein.

The presence of biotin in intestinal contents indicated that it is synthesized by intestinal bacteria; that when rats are fed raw egg white the avidin combines with the biotin formed by these bacteria and the result is a biotin deficiency which manifests itself in the skin disease described by György. This is further confirmed by the fact that failure to include biotin in the rat's diet does not produce the disease unless the diet also contains avidin. In the absence of avidin, the intestinal bacteria can produce the rat's biotin.

Biotin is one of the most powerful growth stimulants yet discovered. Kogl reported that the amount necessary to add to a 2 ml. culture to cause 100 percent increase in growth is only 0.00004 micrograms. Its action is still evident in a concentration of 1/400,000,000,000.

Although biotin, which is essential for yeast and other organisms, has received considerable attention, it is not yet known just how this factor acts in metabolism. It may function as a respiratory enzyme. It increases the oxygen consumption of rhizobium. It increases the fermentation of yeast more markedly than its respiration and its respiration even more immediately than its growth. Addition of biotin to yeast increases both aerobic and anaerobic respiration perceptibly in a few minutes—manifold in a period of hours. For these actions, available nitrogen is necessary—ammonia is probably the best source. The universal distribution of biotin and its presence in quantity in seeds suggests that it may have very general function.

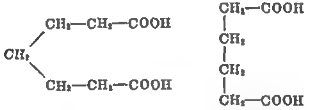
Recent experiments of du Vigneaud and coworkers with the Allen strain of the diphtheria bacillus indicate that pimelic (see Fig. 29) acid is utilized

for the synthesis of biotin, but pimelic acid is unable to replace biotin in its growth stimulating effect on yeast.

R. E. and E. Eakin (1942) have produced further evidence that pimelic acid promotes the synthesis of biotin but that the lower homologues of pimelic acid (succinic, glutaric and adipic acids) and an isomer, beta-methyl adipic acid are inactive. The higher homologues, suberic and azelaic acids, possessed activity comparable to that of pimelic acid.

As early as 1929 it was suggested that the biotin deficiency syndrome in rats was analogous to erythredema (Swift's or Pink Disease) in children and the low content of biotin in human milk was suggested as explanation of the high incidence of Liner's disease in breast fed infants.

Special interest in biotin was aroused by finding it to be especially concentrate in cancer tissue. Rats in which liver cancer was produced by the feeding of butter yellow were protected against its effects if riboflavin and casein were added to the ration but unexpectedly, the protective effect of the diet was destroyed by the addition of biotin Attempts have been



Pimelic Acid Adipic Acid

FIG. 20. PIMELIC AND ADIPIC ACIDS

made to delay cancer growth by feeding raw egg white or avidin with the idea that it might combine with the biotin in the cancer tissue and render it inactive.

PARA-AMINOBENZOIC ACID (P.A.B.) (PABA)

In 1941 Ansbacher reported that para-aminobenzoic acid must be considered a vitamin and a member of the B-complex group. Ansbacher also showed it corrective of hair greying in rats and in the same paper also stated:

> "The experiments to date seem to permit the conclusion that p-aminobenzoic acid is one of the factors of the B complex."

To date, para-aminobenzoic acid (Paba for short) is said to have the following functions: It is a growth factor for rats, chicks and yeasts and an essential ingredient for bacterial culture media. It nullifies the bacteriostatic effect of chemotherapeutic agents of the sulfonamide group and has antigrey hair activity in the nutritional and hydroquinone achromotrichia of the rat.

These claims are all supported by experimental evidence. Sieve has demonstrated that it has a corrective action against hair greying in man. Martin (1942) suggested that it cooperates with pantothen in this action and best results come from a certain relative ratio of these two factors in the diet. In the same year Martin reported folic acid to also be an achromotrichial factor and in the same paper suggested that the effect of Paba on growth of rats is due to its effect on the growth of vitamin producing intestinal bacteria; that Paba plays its major rolé in altering the flora of the intestinal tract.

Martin, Wisansky and Ansbacher (1941) reported that Paba modifies the formation of melanin. Bloch (1917) considered melanoblast pigmentation to be due to a specific "dopa" oxidase which caused formation of melanin by action on dopa (3,4-dihydroxyphenylamine); sulfanilimide has a similar effect but calcium pantothenate has no such influence.

The action of Paba on melanin formation may be the explanation of its role in control of hair coloring, an action quite different from that of Pantothen. According to Sieve, 100 mgm. T.I.D. give the best results with human subjects in hair color control. It affects both hyper- and hypopigmentation of skin. It is non-toxic, at least in amounts many times higher than Sieve used it. According to Wiedling it is of significance in the metabolism of both animals and plants.

FOLIC ACID

In 1941 Mitchell, Snell and Williams reported the separation of a concentrate from spinach to which they gave the name "folic acid." It was adsorbable on charcoal and Lloyd's reagent.

Folic acid was originally defined by its discoverers as the active principle required for the growth of *streptococcus lactis* R., under specified conditions.

Mitchell et al. (1944) claim the concentration of folic acid from spinach to a high degree of potency and have suggested the formula $C_{15}H_{15}O_8N_6$ as the probable composition. It has, however, not yet been isolated in pure form and there is definite indication today that sources of folic acid assayed microbiologically by response of *S. lactis* R. may contain more than one nutrilite; that the factor which stimulates the growth of *S. Lactis* R. may be different from that which acts on *L. casei*.

Mitchell et al. claim for folic acid:

(a) activity in growth promotion of four strains of yeast.
(b) essential to growth of *L. casei*; *L. debruckii*; *clostridium telani* and stimulatory to *L arabinosis*.

L. Casei Eluate Factor

In 1940 Snell and Peterson first postulated what has come to be called the *L. casei* eluate factor. It was because the spinach folic acid concentrate

also stimulated *L. casei* growth to the same degree as the growth of *S. lactis* R. that casei-eluate factor and folic acid were at first believed identical. Further studies using these two different bacterial test organisms and different source materials has changed this viewpoint.

For example, Luckey, Briggs and Elvehjem using solubilized liver preparation as a standard for comparison report these results:

Nutrilite source	*Activity on (relative)* *S. lactis R.*	*L. casei*
Solubilized liver	100	100
Whole liver substance	186	68
Grass juice powder	147	68
Dried grass juice powder	11	29

And Hutchings et al. have reported further evidence to show that the factor that stimulates *S. lactis* may be different from that acting on *L. casei*. Their standard for comparison was a crystalline product separated from liver. This crystalline product produced half maximum growth with *L. casei* at a concentration of 0.000055 micrograms per ml. of culture medium and half maximum growth of *S. lactis* R. at a concentration of 0.00025 micrograms per ml. of culture medium. Using these two organisms in microbiological assays, they report these differences in behavior of various sources:

A. Products with the same relative potency of Liver Standard

Products	γ/gm.
Soy bean meal	3.37
Oats	0.45
Fish meal	0.30

B. Products more active for *S. lactis* than for *L. casei*

Products	*Assay by* *L. casei* γ/gm.	*S. lactis R.* γ/gm.
Wheat	0.55	1.20
Polished rice	0.08	0.13
Ground yellow corn	0.02	0.20

C. Products more active for *L. casei* than for *S. lactis* R.

Products	*Assay by* *L. casei* γ/gm.	*S. lactis R.* γ/gm.
Alfalfa meal	2.61	1.72
Yeast	2.20	1.00
Rice bran	1.60	0.86
Linseed meal	1.30	0.39
Bone marrow	0.97	0.325
Meat scraps	0.77	0.26
Egg yolk	0.62	0.425
Whey	0.36	0.10
Egg white	0.179	0.088

Keresztesy, Rickes and Stokes (1943) tested a sample of Mitchell, Snell and Williams' folic acid concentrate comparing its action on *S. lactis* R. and *L. casei* with various types of extracts and liver preparations. They found the spinach extract to have the same degree of activity for both organisms but preparations from other sources more active for *S. lactis* R. than for *L. casei*. They report the isolation of one factor which they believe to be a new nutrilite that was 2500 times as active for *S. lactis* R. as for *L. casei*.

Further evidence that L. casei eluate factor and folic acid are not identical. In 1938, Stokstad and Manning presented evidence that chicks required for growth a factor different from any vitamins then known and to which they gave the tentative designation of Factor "U". In 1939, Hogan and Parrott also demonstrated a new factor necessary for chick growth and also preventive of a severe form of anemia. This product they called vitamin "B_c". And as early as 1935, Day and associates had postulated a vitamin "M" present in liver extracts and curative of a nutritional cytopenia in monkeys.

Hogan and Parrott's B_c has been isolated from liver by Pfiffner and associates. It has been demonstrated to have the following properties; properties identical with those of crystalline products isolated by Stokstad and by Hutchings et al and shown to be identical with the *L. casei* factor of liver.

Properties of "B_c".

(1) The crystalline B_c isolated by Pfiffner et al. from liver has the following percentage composition:

C, 50.50, 50.63; H, 4.78, 4.78; N, 19.91

and Stokstad gives the composition of his methyl ester as:

C, 52.7; H, 4.8; N, 20.1.

These data indicate identity of Stokstad's product and Pfiffner's B_c and do not indicate identity with folic acid if Mitchell, Snell, and Williams' hypothetical $C_{15}H_{15}O_8N_6$ is the true composition of "folic acid."

(2) The free acid and its methyl ester crystallize as small needles or threads; the barium salt in needles.

Loses activity rapidly when autoclaved in acid solution; more stable in alkaline solution.

Labile to reducing agents but more stable to oxidizing agents.

Insoluble in common organic solvents except for glacial acetic acid, phenol, hot methanol, and formamide.

N.B. Mitchell and Williams reported their "folic acid" solubility in water to be limited, [illegible] mg./ml. @ 30° and 1 mg./ml. at 0°; slightly soluble in glacial acetic acid and

liquid ammonia; essentially insoluble in dry methanol, ethanol, butanol, acetone, ether, dioxane, benzene, petroleum ether, and chloroform.)

Precipitable by heavy metals in varying degree. Zinc best for selective precipitation. Not precipitated by common basic precipitants such as ammonium rhodanilate, ammonium reineckate, flavianic, picrolonic or picric acids.

More strongly adsorbed on fullers earth and norite from acid than from neutral or basic solution; removable by elution. Also adsorbable on aluminum oxide and hydroxide and anthranilic acid but difficult of recovery. Adsorbed by various metallic sulfides.

Activity not destroyed by pepsin, trypsin, ficin, yeast peptidase or phosphatase.

At present, as a result of comparisons of crystalline B_c with hitherto postulated factors; factor U, Vitamin M, Vitamin B_c and *L. casei* eluate factor appear to be identical but not identical with "folic acid."

This viewpoint is based on the following evidence:

(a) All of them stimulate the growth of *L. casei.*
(b) All of them are growth factors for chicks.
(c) All of them are anti-anemia factors for the chick, and
(d) The *L. casei* factor does correct nutritional cytopenia in monkeys.

Possible relation of B_c, L. casei eluate factor, and folic acid to xanthopterin. Mitchell and Mitchell and Williams (1944) basing the observation on absorption of ultra violet light by impure solutions of folic acid suggested the presence in folic acid of a structural unit similar to xanthopterin (see Fig. 12)

Snell and Peterson (1940) suggested that in certain properties the *L. casei* factor resembled a purine. Stokstad reported guanine and thymine to have *L. casei* growth stimulating power and Stokes (1944) reported that thymine could replace folic acid in stimulation of *S. lactis* R. but the amount required was 3000 times that of folic acid; that for maximum growth of *S. lactis* R using either thymine or folic acid, adenine must also be present. That guanine or xanthine were less effective than adenine.

Wright and Welch reported a chemical relationship between *L. casei* factor and xanthopterin (see Fig 12). They found that urine, autoclaved grass, and liver extracts contain a substance which appeared to participate in the synthesis of *L. casei* factor by rat livers. Reactions indicated that this substance might be xanthopterin and when synthetic xanthopterin was incubated with liver, more *L. casei* factor was found by microbiological assay than accountable on basis of the liver's original activity.

Wright, Skeggs, and Welch (1944) suggest that xanthopterin takes part in a metabolite-antimetabolite relationship or mass action inhibition of enzymic activity. Wright and Skeggs (1944) report that when xanthop-

terin was added to cultures of *A. aerozenes*, only small amounts of *L. casei* factor were synthesized by the organism and suggest that xanthopterin may partially replace *L. casei* factor in metabolism or its structural similarity to an hypothetical intermediate in the microbiological synthesis of the factor enables it to inhibit the synthesis or utilization of the intermediate.

Totter and Day (1943) reported that xanthopterin was not only partially effective against monkey cytopenia but also capable of replacing folic acid in rats fed succinyl sulfathiazole. Wright and Welch could not confirm this but suggest that the difference in their results might be due to age of animals and duration of feeding; that possibly the conversion of xanthopterin to *L. casei* factor or folic acid requires the presence of a second factor which might be limiting under certain conditions, a relation analogous to intrinsic and extrinsic factors in the production of antipernicious anemia factor.

Tschesche and Wolf (1937) claimed xanthopterin effective in cure of rat anemia produced by feeding goat milk. Norris reported that a 75,000 potency folic acid concentrate was one-fifth of the activity of xanthopterin in prevention of anemia in trout.

Other nutrilites possibly members of "folic acid" complex. Recently Briggs et al. have reported two vitamins designated B_{10} and B_{11}, necessary for the feathering and growth of chicks and not replaceable by *L. casei* factor though having certain properties in common when measured by *L. casei* or *S. lactis* R. response.

Known L. casei eluate factor requirements

Bacteria for growth: *L. casei*, *Lactobacillus helviticus*, *Profionibacterium pentoseaceum*, *S. lactis* R., Tetanus bacillus and Tetanus toxin.

Chicks: for growth and anemia prevention.

Rats: for growth.

Guinea pigs: for growth.

Monkeys: for prevention of leukopenia and anemia.

Dogs: for prevention of normocytic anemia.

Relation to human needs not yet satisfactorily evaluated.

CHOLINE

As stated in Chapter II, choline received consideration as a possible vitamin with the discovery of its lipotropic action. In 1939 Best and Ridout pointed out that the fatty liver could be prevented by feeding choline. Schaefer et al. reported severe anorexia and growth failure of puppies on a low choline diet. The following results of choline deficiency have been reported by various investigators:

(a) Essential to normal nutrition and egg production of chickens and young turkeys and preventive of slipped tendon disease

(b) Preventive of renal hemorrhage.
(c) Preventive of liver necrosis, cirrhosis and cancer resulting from addition of "butter yellow" (dimethyl-amino-azo-benzine)
(d) Necessary to normal lactation and nutrition of rats.
(e) Depresses the polycythemia induced by cobalt
(f) Essential to formation of acetylcholine, the impulse transmission essential of nerve endings and synapses. Nachmanson and Muchado have extracted an enzyme, choline acetylase, necessary for the formation of acetylcholine from rat brain. It is suggested that acetylcholine liberation is responsible for transmission not only across synapses but along all nerve pathways.

Best writes of the development of his interest in choline as a factor in prevention of fatty livers (1941). He states that insulin treated animals were observed to often show large yellow livers. This condition could be prevented by inclusion of raw beef pancreas in the diet and *also by feeding lecithin*. Later, he and coworkers proved that it was the *choline* fraction of the lecithin that accomplished the prevention of the fatty infiltration. Later still, Ridout and Channon showed that choline accelerated the removal of cholesterol esters as well as neutral fat from the liver.

Dragstedt has postulated a lipotropic factor in pancreas which he calls "lipocaic" which he maintains is not choline and is a second internal secretion of the pancreas.

The lipotropic action of choline reported by Best stimulated study of its mechanism and du Vigneaud and associates have contributed to that solution by proof that one, at least, of its principal roles is to supply labile methyl groups. This viewpoint was indicated by proof that accumulation of excess fat in liver could be prevented either by feeding choline or betaine or by methionine or a protein containing this sulfur amino acid. When Griffith observed that choline deficiency could also produce hemorrhagic degeneration of the kidney, it was shown that this condition also could be prevented by methionine or betaine. Pyridine poisoning of rats can also be counteracted by inclusion of methionine or choline and homocystine in the diets, that pyridine in its detoxication is methylated to form N-methyl pyridinium hydroxide.

Let us first consider these three effects of choline deficiency:

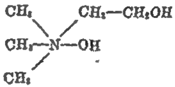
(a) Growth failure
(b) Fat accumulation in the liver
(c) Kidney hemorrhage

How does choline function to correct each of these conditions?

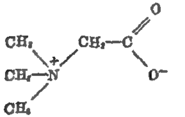
According to du Vigneaud transmethylation is an important metabolic function; it is dependent upon a supply of compounds with labile methyl groups. Choline, methionine and betaine all contain such groups (see Fig. 30).

It was shown that feeding cystine or decreasing methionine aggr the effects of a choline deficient diet but when *choline and cystine we* bined the combination was protective, as were a combination of choli homocystine or methionine alone. du Vigneaud showed that:

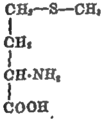
(a) Supplements of choline or betaine to a diet which is devoid of met *and cystine stimulated growth of young rats owing to the forma* methionine, an essential *amino acid, from homocystine and a methy* supplied by the choline or betaine.



(a) *Choline*



(b) *Betaine*



(c) *Methionine*

FIG. 30

(b) That isotopic hydrogen in the methyl group of *methionine adminis* to rats could be isolated later from tissue choline and creatine.

(c) *That the methyl groups of creatine and* sarcosine are not labile, since ne compound permits growth when added to a methyl-deficient diet con ing homocystine.

Choline then can supply the methyl groups with which to form met nine and Rose has shown that this is an *essential* amino acid, which n be supplied or growth fails. According to Griffith cystine and choline not regarded as antagonistic. Increase of cystine and its stimulatio growth simply creates a greater need for choline.

How does choline, betaine or methionine cause fat to move out of the liver?

According to Bloor, fat is mobilized in the liver which is a fat storage organ. Of this fat, a considerable portion and perhaps all of it is changed to phospholipid, of which a large part is thus able to be discharged into the blood and be distributed to other tissues. For phospholipid formation, choline is necessary (to form lecithin phospholipid). Choline deficiency prevents lecithin formation and hence ability of storage fat to pass into circulation. Methionine supplies labile methyl groups from which to build choline. Choline may also promote the utilization of the fats in the liver. The explanation of how choline or methionine prevents renal hemorrhagic degeneration is not so clear. It may be because methylated compounds or methyl contributing substances such as phospholipids or other substances are essentials to kidney cell organization as well as to growth and to lipid metabolism.

FORMATION OF CHOLINE IN THE BODY

Stetten suggests that ethanolamine is the precursor of choline in the following reaction:

$$\begin{array}{ccccccc} \text{Betaine} & \longrightarrow & \text{Glycine} & \longrightarrow & \text{Ethanolamine} & \longrightarrow & \text{Choline} \\ \downarrow & & & & + & & \\ \text{Methyl} & & & & \text{Methyl} & & \end{array}$$

CHAPTER XI

THE NATURE AND FUNCTION OF VITAMIN C

In 1757 James Lind published his classic "Treatise on Scurvy," the first clear account of the disease and established the efficacy of lime and lemon juice.

In 1904 Sir Gilbert Blaine secured regulations enforcing supply of lime juice to the sailors of the British Navy and in 1865 similar regulations were adopted for the mercantile marine.

Funk, in his first review of possible avitaminoses, suggested that scurvy might be a vitamin deficiency disease but Holst and Frölich (1912) initiated modern research for identification of the antiscorbutic factor by producing it experimentally in guinea pigs. Höjer's work greatly extended this earlier work of Holst and Fröhlich and especially the relation of the antiscorbutic factor to tooth formation.

The characteristic of the vitamin that proved the best clue to its nature was its instability. This was shown by the work of Zilva in England who contributed much to the chemical identification of the vitamin and to knowledge of its properties. In his studies and in those of Bezssonoff in France and of Vedder and King in America, it became increasingly evident that the vitamin was a compound whose activity was rapidly destroyed by oxidation.

This was further confirmed by studies of the methods of preserving antiscorbutic foodstuffs. Commercial canning was shown by Eddy and Kohman (1924–1925) to owe its protective action against vitamin C destruction to control of oxidation.

In 1922 Zilva showed that decitrated lemon juice lost 80 percent of its antiscorbutic potency in one half hour if made N/20 alkaline and exposed to air at room temperature. No loss of potency occurred if air was excluded. This was confirmed by Kenny in Sherman's laboratory in 1926. Studies that have followed this early work have further confirmed the fact that the primary cause of vitamin C destruction is oxidation; that heat accelerates the oxidative destruction and cold retards it, that destruction is more rapid in products with alkaline reaction and less rapid in those with acid reaction.

Progress toward the isolation of the vitamin was retarded from 1916 to 1920 by the infection theory. Jackson and Moore recovered from guinea pigs made scorbutic on a diet of oats and milk, a diplococcus which they suggested might be the etiological factor. At that time oats and milk were supposed to supply all the nutrient factors needed by the guinea pig.

The following year, McCollum and Pitz reported studies that appeared to confirm the infection theory. They produced scurvy in guinea pigs on an oats and milk diet as Jackson had done and McCollum described their attitude at the time in these words: "They found it difficult to believe that the disease could be due to the lack of a specific substance, for milk alone suffices as the sole food for all young mammals during a critical period of their lives." Also examination of their oats and milk fed guinea pigs showed the cecum distended with impacted feces which condition appeared to support the infection theory.

Shortly after the publication of McCollum and Pitz' findings, Chick and Hume in London and Mendel and Cohen in America again produced evidence supporting the view that scurvy is of dietary deficiency origin and not an infection. Chick and Hume showed milk to be far less protective against scurvy than had been assumed and Mendel and Cohen that by feeding a superior diet, they could still get scurvy but without any sign of impacted feces.

A few years later Parsons furnished the final explanation of McCollum's inconsistent results. Parsons found that while oats and milk constituted a nonscurvy diet for rats, the reason was due to the fact that unlike the guinea pig and the human species rats are able to synthesize this factor. This was one of the first studies to reveal that certain animals synthesize enough of a vitamin to make supply from food unnecessary; the first study to suggest that in determining vitamin requirement we must always take into account amounts and kinds produced by the test animal. It changed our definition of a vitamin as something that must always be supplied exogenously

Parsons proved her point by showing that the livers of rats reared on a diet that produced scurvy in guinea pigs contained enough of the antiscorbutic factor to make those livers protective when fed to guinea pigs on the oat-milk diet. These findings were confirmed by Lepkovsky and Nelson.

The attempts to isolate the antiscorbutic factor were then resumed. In 1930–32 two chemists at Frankfurt, Germany, made a contribution that materially speeded up progress to final identification of the antiscorbutic factor. They had occasion to distinguish between fresh and stale and true and artificial fruit juices. They found a distinction could be made by using an oxidation-reduction indicator known as 2.6-dichloro phenol-indophenol. Fresh and true fruit juices gave a strong reaction with the indicator, stale and artificial juices less or no reaction.

Zilva for a time misinterpreted these findings. He found, in agreement with Tillmans and Hirsh, that antiscorbutic juices do bleach the reagent but did not always parallel the estimation of C by animal tests. From such comparisons Zilva drew the conclusion that vitamin C itself does not

reduce the indicator, but that decolorization of it is due to a reducing substance closely associated with the active principle, which tends to protect it against oxidation.

Pure ascorbic acid had not yet been isolated. Tillmans maintained that Zilva was wrong, that it was the vitamin itself that bleached the indicator, that the indicator could be used to measure the concentration of the vitamin and that the vitamin might be hexuronic acid. He also first suggested that partially oxidized the vitamin was more prone to destruction by further oxidation than in its orginal reduced form.

Though Tillmans did not know it at the time, Szent-Györgyi had already demonstrated the reversible oxidation of hexuronic acid but his proof of its identity with ascorbic acid developed later.

What we know of the chemical nature of vitamin C today is reviewed in Chapter II, also the part played in its elucidation by Waugh and King in America and Szent-Györgyi in Europe. Also the proof by synthesis furnished by Reichstein et al. (1933) and by Haworth et al. (1934). The great value of the phenol-indophenol reagent was that it eliminated time consuming animal tests in detecting and measuring vitamin C content. The behavior of ascorbic acid toward the indicator and the successive products of its oxidation are shown in Figs. 13 and 14.

The term hexuronic acid was abandoned in favor of ascorbic acid which is now the generally accepted name for vitamin C both in America and Europe. For a time the Council of Pharmacy objected to the functional designation and the term "cevitamic acid" was in use in the United States.

TITRATION BEHAVIOR OF ASCORBIC ACID

From the reaction shown in Fig. 13, it is evident that titration with phenolindophenol gives us only the amount of l-ascorbic acid in the solution. The amount of the "dehydro" form which can be reversibly changed to l-ascorbic in the animal and human body and thus used to prevent scurvy is not revealed by such titration. However, it is possible, in vitro, to convert the dehydro form to the l-ascorbic form by use of reagents such as H_2S in acid solution or by cysteine and glutathione and the fixed SH groups in these amino acids and in proteins containing them.

Hence it is now common practice in assaying foods for vitamin C content to first titrate the extracts with the indicator and record the l-ascorbic acid found. Then to treat with H_2S in acid solution and titrate again. The difference gives the dehydro ascorbic content and the sum of the two values the combined l-ascorbic and dehydroascorbic acid content, i.e., the total antiscorbutic potency.

In body fluids such as blood, urine, cerebrospinal fluid the ascorbic acid present is apparently almost completely in the l-ascorbic form, eliminating

the need for the procedure for dehydroascorbic acid content. (Borsook; Farmer and Abt, 1936 and 1937).

In urine titrations, however, according to Evelyn there may be present substances other than ascorbic acid which will react with the indicator. pH control eliminates some of these factors and there is a differential in speed of action between the indicator and ascorbic acid and the other reducing substances, ascorbic acting faster.

PROTECTING ASCORBIC ACID STABILITY

It is obvious from the preceding that loss of ascorbic acid can be prevented by preventing oxidation and accelerated by any factor or conditions favorable to oxidation. It is further realized that with access to oxygen or oxidizing reagents, raising temperature will accelerate ascorbic acid inactivation and lowering temperatures will tend to retard it. It is also obvious that since ascorbic acid is readily soluble in water it can be more or less readily leached out of a source material and prolonged soaking will increase such losses.

All of these findings have had definite effect on the handling of foods containing vitamin C and the preservation of their antiscorbutic potency.

Table XXIII illustrates some of the findings related to household practice and marketing and storage of antiscorbutic foodstuffs.

The pure vitamin in solid form is quite resistant to oxidation compared to its behavior in solution and for that reason vitamin tablets containing the solid ascorbic acid remain stable for long periods of time.

OTHER COMPOUNDS WITH ANTISCORBUTIC POTENCY

l-Ascorbic and dehydroascorbic acid are not the only compounds with antiscorbutic potency but they appear to be the principal forms in food and biological materials. The following have been synthesized and tested for antiscorbutic value with the results noted:

l-rhamnoascorbic acid	$\frac{1}{5}$ potency of l-ascorbic acid
l-araboascorbic acid	$\frac{1}{20}$ potency of l-ascorbic acid
l-glucoascorbic acid	$\frac{1}{40}$ potency of l-ascorbic acid
l-galactoascorbic acid	$\frac{1}{100}$ potency of l-ascorbic acid
d-ascorbic acid	no potency
d-glucoascorbic acid	no potency
d-galactoascorbic acid	no potency

It would appear that one essential to antiscorbutic activity is associated with the position of the lactone bridge; the d-forms inactive, the l-forms active. That the ring position is not the only factor involved in antiscorbutic action is evidenced by the fact that the two *l* forms listed above do not have the potency of l-ascorbic acid itself.

TABLE XXIII

Effect of Food Handling on C Content

A. *Storage Losses*

Variety of Spinach:	Storage Temp. in degrees Fahrenheit	Mgm. ascorbic per gm. after 0 days	3 days	7 days	17
Hollandia.	34–38	0.78	0.76	0.72	0.
Hollandia.	74–83	0.78	0.44	0.05	sp

B. *Cooking Losses*

Cabbage	Per cent of original ascorbic acid in drained cabbage	in cooking water	destroye
Boiled finely shredded.	22 ± 0.6	66 ± 0.5	12 ± 0.
Boiled finely strips	32 ± 1.3	53 ± 1.1	15 ± 1.
Boiled finely quarters	38 ± 1.1	56 ± 1.5	16 ± 2.
Steamed finely shredded	24 ± 1.1	50 ± 0.4	26 ± 0.
Steamed quarters	58 ± 1.1	22 ± 0.3	10 ± 1.
Panned finely shredded	66 ± 1.3	None	31 ± 0.

C. *Losses on Holding*

	m p
(1) Potatoes in Fall (Netted Gem Var.)	0
After 6 mos. storage in cool dry storage.	0
After 6 mos. storage in warm dry storage	0
Potatoes in Fall (Bliss Triumph Var.).	0
After 6 mos. in cool dry storage	0
After 6 mos. in warm dry storage	0

(2) Apple Pie

After baking	0.022 mgm. per gm. of C = 80% loss
48 hrs. after baking	0.013 mgm. per gm. of C = 88% loss

(3) *Steam Table Losses*	*Loss on standing on steam table 3 hours*
String beans	61.4% on dry basis
Broccoli	63.5% on dry basis
Cabbage.	91 0% on dry basis
Carrots	91.4% on dry basis
Cauliflower	64.0% on dry basis
Spinach	96.0% on dry basis
Potatoes	26.5% on dry basis

D. *Losses Due to Metallic Catalysis*

Plant ascorbic acid oxidase claimed by King to produce action because o

It is evident from the reaction of ascorbic acid shown in Figs. 13 and that it is capable of acting as a hydrogen acceptor and that the action reversible (*see also Chapter III*). But, as previously stated, it has ne

been shown to act as the prosthetic group of a respiratory enzyme. Its reducing action is due to the endiol group $\begin{array}{c}-C-OH \\ \| \\ -C-OH\end{array}$.

HOW IS ASCORBIC ACID PRODUCED?

Vitamin C is l-xyloascorbic acid and in the laboratory it is synthesized by starting with xylose or sorbitose as a base. Extensive studies have been conducted with plants to explain its biosynthesis which we may review briefly.

Vitamin C in plants is found mainly in the green tissues and especially in the leaves of plants which suggests a relation to photosynthesis. However, while seeds as a rule contain no vitamin C they are able to produce it by germination before photosynthetic organs are developed. The findings of Harris and Ray, for example, are shown in Table XXIV.

TABLE XXIV

Effect of Germination on C Production

(Acc. to Harris and Ray, 1931)

	CONTAIN
	mgm C/gm
Pisum seeds before germination	0
After soaking 24 hours, no germination	0.03
Germinated 24 hours	0 69
Germinated 72 hours	0.82
Germinated 96 hours	0.86

What is the precursor in this process?

Von Hausen showed that it must be a carbon source in the mineral medium since excised embryos after removal of the cotyledons require ascorbic acid in the culture medium in early stages of growth but later are able to synthesize the vitamin. This carbon source is probably sugar because Ray showed that mannose in the culture medium permitted abundant formation of ascorbic acid by excised embryos. This view is further supported by the fact that animal organs such as spleen, liver, and cardiac muscle are able to synthesize ascorbic acid from mannose in vitro. Apparently the mechanism is enzymatic and the enzyme a dehydrogenase. The mannose is probably first changed to xylose.

But what of the relation to photosynthesis? Why abundance in green parts of plants?

Reid (1937) claims its formation is quite independent of chlorophyll and that its greatest accumulation is in actively growing organs, particu-

larly in the regions of elongation, in leaf buds and floral buds. T reason for association with chlorophyll might be because it requires su that is formed by photosynthesis and hence its formation and accumulat naturally occurs in the same place as the site of sugar formation. that is the case it is important to find just when after photosynthesis sugars, ascorbic acid formation begins and how. That question has r yet been answered.

There are other factors influencing ascorbic acid beside the supply sugar. Red light favors the action. Manganese appears to also fav ascorbic acid formation. And certain mineral salts, notably, K $Ca_3(PO_4)_2$ and $Ca(NO_3)_2$ help, perhaps by influence on carbon and nitrog metabolism. All these point to an enzyme system but its components a still unexplained.

HOW DOES ASCORBIC ACID FUNCTION?

There is definite evidence that it is a growth factor for certain micr organisms but it is not certain that growth stimulation and antiscorbut action are due to the same structural elements. For example, d-gluc ascorbic acid has no antiscorbutic effect on animals but has a defini growth stimulating effect on certain flagellates. It is also uncertain wheth the growth effect is catalytic in nature or due to its use, like sugar, as nutrient.

The fact that it can undergo reversible oxidation, act as hydrogen a ceptor and donor; that its oxidized form can be reduced by SH compoun such as glutathione and proteins containing the SH groups naturally su

prosthetic group in such an enzyme system.

Szent-Györgyi first isolated the vitamin from adrenal cortex and wa interested in it as a factor in oxidation; in vegetable respiration. It wa only later that he realized that he had the antiscorbutic vitamin. Szent Györgyi knew that persons suffering from a deficiency of adrenal corte factor (Addison's disease) developed a brown pigmentation. Also tha "dying" bananas, apples, and potatoes also show a brown discoloration He first studied the vegetable browning process. In that study he foun the brown discoloration to be due first to formation of quinones fron catechol by removal of 2 hydrogens and that these yellow quinones if no at once reduced combined with proteins and amino acids in the tissue to form the so-called melanoid pigments. Associated with catechol in plan tissue he found an enzyme. When the plant was damaged this enzyme caused a rapid oxidation of catechol to quinones and the plant lost its ability to reduce these quinones, resulting in the formation of pigments

This enzyme system is known as the polyphenoloxidase system and Kubowitz and Keilin have shown that catechol oxidase is a copper-protein complex. There is also always present with catechol oxidase another enzyme, a peroxidase containing iron. This peroxidase reacts with the H_2O_2 formed by the catechol oxidase and with peroxide oxidizes another molecule of catechol. In this way the hydrogens of the catechol are converted to water. As Szent-Györgyi says:

"The catechol oxidase system was thus cleared up but it gave no clue to the function of the adrenal cortex. It is true that the catechol derivative of the polyphenoloxidase of plants is closely related to adrenalin, but it was the cortical part of the adrenal gland that primarily interested me. So, obtaining no answer to my question from the polyphenoloxidase system and the plants that discolored on dying, I turned to the plants that did not turn brown. I switched the question around and asked no more why patients with defective adrenals turned brown, but why it was that we, who had good adrenals, remained colorless. . . . Very soon I found that many plants that belonged to this group not only failed to turn brown themselves, but their juice, if added to the juice of polyphenoloxidase plants, prevented these from coloring for some time . I found that the reason lay in the presence of a most fascinating substance which can reduce all quinones to phenol and in this way prevent pigment formation, in other words, the substance had two labile hydrogen atoms which it very readily gave up to quinone. . Eventually, I crystallized it, that is to say, peeled it out in a pure condition "

This quotation explains Szent-Györgyi's approach to what was then called hexuronic acid, and its presence in adrenal cortex. Starting with this product Szent-Gyorgyi's two labile hydrogens are now known to be the endiol group $\begin{matrix} -\text{C}-\text{OH} \\ \| \\ -\text{C}-\text{OH} \end{matrix}$. His interest was further stimulated by the fact that ascorbic acid dosage bleached out patients with Addison's disease. He did not find in ascorbic acid the solution he sought of the function of the adrenal cortex but he discovered some data bearing on the possible operation of ascorbic acid. He found in cabbage an enzyme, ascorbic acid oxidase, which, like polyphenoloxidase, had a copper-protein structure with copper the prosthetic group and also an associated peroxidase. This system's action on ascorbic acid differs from that of the polyphenoloxidase system in the following way, according to Szent-Györgyi. In the polyphenoloxidase-peroxidase system the peroxidase is not able alone to effect an oxidation of ascorbic acid but requires an intermediary phenolic substance. The peroxidase oxidizes the phenol which then, in turn, oxidizes the ascorbic acid. The specific phenol in this case appears to be benzopyrane, a flavone.

It is obvious from the above discussion that the ascorbic acid oxidase-peroxide-phenol flavin system could be the culmination of a metabolic

ascorbic acid in prevention of capillary bleeding. Of this discovery and choice of name, Szent-Györgyi writes as follows (1939):

"In citrus fruits we found a specially active member of this group (flavanols) present as a glucoside which up to that time had been unknown in this form. We called it with V. Bruckner 'eriodictin.' . . . In the unripe plant we find this substance in a methylated, inactive, stable form which has been known for a long time as hesperidin."

and again:

"I had a letter from an Austrian colleague who was suffering from a severe hemorrhagic diathesis (vascular type). He wanted to try ascorbic acid in his condition. Possessing at that time no sufficient quantities of crystalline ascorbic acid, I sent him a preparation of paprika that contained much ascorbic acid and the man was cured by it. Later with my friend, St. Rusznyak, we tried to produce the same therapeutic effect in similar conditions with pure ascorbic acid but we obtained no response. It was evident that the action of paprika was due to some other substance present in this plant. It would have been a hopeless job to try and find and isolate this substance had we not had our experience with flavons. So we set out to prepare flavons, in the first place eriodictin, that can easily be injected and we found that similar pathological conditions, not previously amenable to therapy, could be cured by it with regularity The effect had several characteristics of vitamin action, so, tentatively, I called it "Vitamin P" in honor of Paprika and Permeability, on which later it was found to have an influence. As yet, I have failed to demonstrate its vitamin nature by animal experiments and until such proof is given, the vitamin nature of this substance is not beyond doubt."

The active fraction of Szent-Györgyi's Vitamin P as extracted from *lemons has been called "citrin." This citrin is a mixture of two glucosides;* an inactive glucoside (hesperidin) and an active glucoside (eriodictin or eriodicytol). For chemical structure and properties see Chapter II., Fig. 20.

Lorenz and Arnold (1939) prepared a solution of citrin suitable for therapeutic use and Kugelmass (1940) has reported the value of citrin in the treatment of vascular purpuras. Kuglemass suggested that as glucosides are recognized as capable of immobilizing substances with potential activity until needed in metabolism or detoxication that perhaps its value in protecting capillary resistance resides in its detoxifying power.

Scarborough (1939) who has supplied evidence of its value to human subjects claims that it protects against capillary fragility and is different from ascorbic acid, its activity apparently directed more to peripheral capillaries.

It is more abundant in the albedo of the lemon rind than in the juice and to date, the lemon rind has proved richer in this factor than other citrus fruits. Its vitamin character and its behavior are still to be established.

CHAPTER XII

THE NATURE AND FUNCTION OF VITAMIN D

The modern investigation of the antirachitic vitamin commenced during the war years (1914–18) and was given great impetus by Mellanby in England who studied the effect of various diets on puppies. He showed that he could produce rickets in these animals by feeding them milk and bread or oatmeal porridge. At that time an emulsion of linseed oil was widely sold in England as a cure for rickets and the casual listing of this product, which was worthless, led to the dietary study of other fats. Cod liver oil was found to be the best antirachitic of the fats tested. Since the mineral values of this stock diet were the same in all these tests, Mellanby was led to the belief that the curative factor was not the mineral element of the diet, a favorite theory at the time, but some peculiar virtue richly present in cod liver oils. It was known that such oils were rich in vitamin A and it seemed logical to assume that this vitamin was the rickets preventive factor (Mellanby, 1921)

The study initiated by Mellanby was followed by five discoveries in particular which, according to Bills, were crucial in leading to our present understanding of the nature of vitamin D.

The first of these discoveries was made by McCollum and associates (1922). They proved that the antirachitic vitamin in cod liver oil was, like vitamin A, present in the nonsaponifiable fraction of the oil but that it was not destroyed by bubbling air through the heated fraction while the vitamin A value was destroyed.

The second of these discoveries was that exposure of certain foodstuffs to ultraviolet irradiation endowed them with the antirachitic effect possessed by fish liver oils. Hess and Steenbock share the credit for this discovery.

The third discovery was that the substance accepting ultraviolet irradiation to become antirachitic was the sterol fraction in the oils and foodstuffs. Hess, Steenbock, and Rosenheim and Webster in England were the discoverers of this fact

The fourth discovery was the identification of the ergosterol of Tanret (1879) as the parent substance of vitamin D. Windaus and Hess and Rosenhiem and Webster are generally credited with demonstration of this substance as provitamin D, though many others participated in compiling the body of proof.

The fifth discovery was the isolation of calciferol, the active component of irradiated ergosterol in 1931 (Askew et al., Windaus et al.).

A summary of the *studies leading up to the demonstration* of ergosterol provitamin D has been made by Bills and as it is concise and compre- nsive we may quote it here:

'Cholesterol of supposedly good purity was found to be activated by irradiation. ore irradiation, it exhibited spectral absorption in the ultraviolet region; after diation, it had *little* or no absorption. In consideration of Beer's Law, one postu- ed that either the cholesterol had been at least half metamorphosed, or else the stance in which the absorption spectrum was changed was a small amount of im- ity which was exceedingly absorptive. It was found that repeated crystalliza- n of cholesterol led to the accumulation of the absorbing fraction in the least ble fraction. Furthermore, the use of very drastic means of purification, such bromination followed by debromination, led to the production of a cholesterol ch apparently had no absorption and no absorbability. The drastic means uded in addition to bromine, such oxidizing agents as permanganate and de- rizing charcoals, and so it appeared that the unknown provitamin might be a hly unsaturated sterol, such as ergosterol.

'Ergosterol was found to be destroyed by the same reagents which destroyed the ubstance in cholesterol. *In one instance, namely in the treatment with* perman- ate, even the rates of destruction of the provitamin and of ergosterol were found be the same. Furthermore, the absorption spectrum of ergosterol was found to sist *of four bands with maxima at 293.4, 282, 270 and 260* $\mu\mu$, which are also maxima of ordinary cholesterol. In ergosterol, the absorption was enormously e intense than in cholesterol, and the vitamin D potency after irradiation was rmously greater. Upon irradiation, the absorption bands of ergosterol under- t changes, and finally faded as did those of the impurity in cholesterol."

For some time after these observations it was believed that ergostero the sole provitamin D; that activation of foodstuffs to antirachitic ency by irradiation would be successful only if ergosterol were present; t fish liver oils owed their activity to the activated ergosterol dissolved hem.

n 1934, Waddell challenged this viewpoint and today we know that the ncipal provitamin D in fish liver oils, associated with cholesterol, is not osterol but another sterol likewise capable of activation by irradiation occurring *in fish oils in the activated state. We are also sure today* t this sterol (7-dehydrocholesterol) and ergosterol are not the only stances capable of acquiring antirachitic potency by irradiation. In , Bills (1938) claims that there are at least ten provitamins D occurring natural sources.

rgosterol and 7-dehydrocholesterol are, however, the most widely ributed forms of vitamin D and when activated to antirachitic potency vitamins so produced are today designated as vitamins D_2 and D_3 ectively; *i.e., vitamin D_2 is activated ergosterol,* vitamin D_3 is activated hydrocholesterol. There is no D_1 in the literature today. What was

first called vitamin D_1 proved to be a mixture of two sterols and the term was discarded when this was discovered. Vitamin D_2 is also known as calciferol. It is generally believed today that the provitamin D present in the human skin and which is activated by exposure to the ultraviolet light from sun or lamp is not ergosterol but mainly 7-dehydrocholesterol.

CHARACTERISTICS OF VITAMIN D

As previously stated, of ten potential vitamins D, two—activated ergosterol (calciferol or D_2) and 7-dehydrocholesterol (D_3) are of importance in medicine today.

ERGOSTEROL

This susbstance is a characteristic sterol getting its name from Tanret's discovery of the substance in ergot in 1879. The commercially available form is today prepared from yeasts and molds in which it occurs to an amount equal to [illegible] per cent of the dry weight of these fungi.

It forms colorless crystals, small in size, whose melting point varies with the water content. The melting point for the nearly anhydrous form is 160°C. Commercial ergosterol usually contains about 5 per cent of an inert sterol which is not activated by ultraviolet irradiation (alpha-dihydroergosterol). Ergosterol is insoluble in water, sparingly soluble in oils, freely soluble in most of the organic solvents. On irradiation only a part of the ergosterol is transformed to calciferol (D_2); ordinarily not more than 50 per cent under the best conditions

During irradiation a series of products are formed which, in order of appearance, are listed by Bills as follows:

Ergosterol
Lumisterol
Tachysterol
Calciferol
Toxisterol (Substance 248)
Suprasterols I and II

Lumisterol, the two supra-sterols and calciferol have been isolated in crystalline state. Tachysterol has been separated as crystalline 3,5-dinitro-4 methylbenzoate. Toxisterol has not been obtained in pure form. Early preparations of irradiated ergosterol which were irradiated too long are believed to have owed their toxic effect to production of this sterol. It is sometimes called substance 248 since it has a single absorption band at 248 $\mu\mu$.

Lumisterol, according to Bills, is probably not antirachitic but is converted into calciferol and it also forms with calciferol a definite addition

product in the ratio of one part lumisterol to one part of calciferol. This combination was the original D_1 of the German investigators.

Tachysterol is also probably not antirachitic and may have a slight toxic effect.

Toxisterol has not been isolated. There is evidence that it is formed more readily when alcohol is the solvent for the solution of ergosterol to be irradiated.

The suprasterols are not antirachitic and are only slightly toxic.

As stated above, it is now possible by control of solvent and irradiation time to convert about 50 per cent of ergosterol into calciferol or active vitamin D_2 without toxic products being formed. When such irradiated ergosterol is dissolved in an inert oil such as peanut or corn oil, the product is known as *Viosterol*. This name was coined by the Council of Pharmacy of the American Medical Association to avoid a multiplicity of trade names by individual drug houses for the same product. When this product was first marketed it was customary to express its vitamin D potency as 250 D or 100 D, etc., by which was meant that it contained 250 or 100 times the amount of D in a certain reference cod liver oil. Today vitamin D potency must be stated on labels in International or U.S.P. units. Viosterol solutions must, however, contain at least 10,000 International units of vitamin D per gram. An International unit is the vitamin D potency of 0.000025 miligrams of calciferol.

The principal commercially available forms of activated ergosterol are irradiated yeast, viosterol, and metabolized milk (milk made vitamin D potent by feeding yeast to cattle).

7-DEHYDROCHOLESTEROL (D_3)

For correction of rat or human rickets, calciferol appears practically equivalent to the vitamin D in cod liver oil. For curing chick rickets cod liver oil has, according to Massengale (1930), something like 100 times the potency of calciferol.

Provitamin D_3, nonirradiated 7-dehydrocholesterol, appears to be the principal form of provitamin D in human skin and in the active form constitutes the major vitamin in fish liver oils such as cod and halibut. It is also the principal form present in vitamin D milks produced by irradiation of the milks and in the vitamin D milks fortified by the addition of cod liver oil concentrates. It is sometimes called the *normal* cholesterol vitamin D since it is the form found in greatest abundance in crude cholesterol.

7-Dehydrocholesterol was obtained in crystalline form by Schenck. Crystalline esters have also been obtained from tuna liver oil and from halibut liver oils. (Brockman, Haslewood and Drummond, Simons and Zucker). Its absorption spectrum is similar to that of calciferol.

OTHER ANTIRACHITIC STEROLS

While D_2 and D_3 are the principal forms of antirachitic sterols in vitamin D containing foods and medicinals there is evidence that others may be present. 22-Dihydroergosterol is found in vegetable products and may be a significant factor in irradiated cereals. It has not yet been isolated from natural products. It appears to be slightly less active for rats, weight for weight, than the other two vitamins and less active for chicks than vitamin D_3 (MacDonald). It was produced in crystalline form by Windaus and Trautmann and has the same absorption spectrum as calciferol.

Activated 7-dehydrositosterol has been studied by Wunderlich and has antirachitic potency. It is present in some vegetables. Its antirachitic potency for rats is less than that of either D_2 or D_3.

Cholesterilene itself cannot be activated to vitamin D potency by irradiation but cholesterilenesulfonic acid and its salts are definitely antirachitic to rats and are more potent for chicks, rat unit for rat unit than cod liver oil. The formula given is that of Stavely and Bergmann. Yoder has described the manner in which it was discovered by treating cholesterol with fuller's earth.

Of the other forms listed, little is known at present and the reader is referred to Bill's reviews (1935–1938).

We have no explanation of how irradiation produces physiological activity. As noted in all the activated forms, the phenanthrene ring appears to be broken at the position 10 and there are double bonds at this point in the ring. There is also, as we have previously cited (Milas and Anderson), evidence that the structure of the side chain enters in some way into determination of activity.

Milas and Anderson have reported success in the synthesis of a compound called triene which has the configuration of the active part of the vitamin D ring and study of such compounds may bring light on this problem.

FUNCTIONS OF VITAMIN D

The function of the vitamin to which we owe its discovery and early study is its ability to prevent and cure rickets. For this study it was necessary to produce experimental rickets in animals and the present U.S.P. test for vitamin D potency depends on this ability and the curative effect on such induced rickets by a given source of D. (For details see Chapter XXVII.)

Sherman and Pappenheimer and McCollum were first to devise rickets producing diets and Steenbock and Black also produced another. All of these produce rickets by a faulty Ca/P ratio in the diet (see Table XXV).

It will be noted that in all of these diets the Ca/P ratio is high. It is therefore evident that rickets can be produced by a faulty Ca/P ratio and, since addition of vitamin D to such a diet cures the rachitic condition, the

lation of phosphatase in the blood. Its presence in the blood is not associated with any function it performs in that fluid and Kay considers it is there because of "leakage" from tissues. On this theory during normal bone formation it operates at the site to increase concentration of PO_4 ions necesary to precipitation. In rickets it escapes from the bones and

TABLE XXVI

Blood Phosphatase in Bone Diseases

(After Kay 1932)

CONDITION	NO. CASES *	AVE. PHOSPHATASE CONTENT OF THE PLASMA
		units
Normals	33	0.14
Infantile rickets	13	1.03
Adolescent rickets	1	2.4 or more
Renal rickets	2	1.20
Fragilitas ossium (infants & children)	6	0.41
Osteitis fibrosa generalized	3	1.8
Osteitis fibrosa local	7	0.21
Osteitis deformans	24	1.7
Osteomyelitis	8	0.27
Arthritis with bony changes	7	0.17

TABLE XXVII

Influence of Vitamin D from Several Sources on the Serum Phosphatase of Chicks

(After Correll and Wise, 1938)

	VITAMIN D PER 100 GMS. OF DIET			
	Phosphatase per 100 cc. of serum using			
	None	18 I.U. as Cod Liver Oil	37 I.U. as Cod Liver Oil	37 I.U. as Tuna Liver Oil
On first day	71.3	71.3	71.3	71.3
In 2 weeks	158.7	56 4	69.6	81.3
In 4 weeks	267.7	44.1	41.4	65.0
In 6 weeks	243 [illegible]	54.8	43.2	115.2
In 8 weeks	240 0	44 0	38.6	76.6

"leaks" into the blood or at any rate, increases in concentration in the blood instead of staying where it is needed.

Kay further supports this theory by suggesting that one method of *D* vitamin functioning is to control the distribution of phosphatase and Correll and Wise' data from chick experiments given in Table XXVII tend to support this view.

The data in Table XXVII certainly show that vitamin *D* does *reduce*

blood phosphatase and also that the form in cod liver oil is more effective than that in tuna liver oil though both are equally effective by rat test (i e., showed same U.S.P. potency).

We have no evidence of vitamin D forming a part of the prosthetic group in phosphatase analagous to the role of riboflavin in a dehydrogenase but these data do show that it can influence the blood content of phosphatase.

OTHER FUNCTIONS OF VITAMIN D

Phosphatases are concerned in combination with redox enzymes in the cellular metabolism of carbohydrates. We have just seen that vitamin D influences in some way their distribution. Is there any evidence that it plays a role in cell respiration?

Presnall has shown that topically appued vitamin D has the power to increase the oxygen uptake of the skin and his findings have been confirmed by Gothlin. Certain skin diseases and lesions have been shown to benefit by use of vitamin D; Dotorsky and Platt in cases of acne; Ceder and Zon in cases of psoriasis. Dalldorf was able to show that the growth of epithelial cells in vitro was stimulated by addition of vitamin D to the culture medium. These effects are cited to suggest that vitamin D may play a role, as yet unexplained, in cell metabolism other than that concerned with bone formation.

If Vitamin D controls all Ca and P metabolism it will obviously play a part in prevention of abnormalities due to faulty utilization of these minerals for other than bone formation in the body. MacBeath has presented evidence to show that it is at least one factor in the prevention of dental caries. It is generally agreed that pregnant women need additional vitamin D to prevent loss of calcium from their bones and teeth.

In celiac disease much undigested fat (steatorrhea) and calcium salts are lost in the feces. Vitamin D by mouth or by ultraviolet irradiation has been reported beneficial in such cases.

Knapp has produced keratoconus in dogs by feeding a diet deficient in both D and calcium.

HOW MUCH VITAMIN D?

The dosage necessary to protect infants from rickets is fairly well established (Table IX). For adults the amount required is still undetermined but it is generally agreed that 400 units per day is adequate.

In ordinary doses it is not toxic. Natural foods contain little or none of this vitamin.

According to Park:

"The rule holds that the dose of vitamin D will not become toxic so long as the calcium and inorganic phosphorus levels in the blood are not affected Apparently the toxic action does not depend upon the level of vitamin D in the blood but rather on its effect on the calcium and phosphorus metabolism."

Chapter XIII

NATURE AND FUNCTION OF VITAMIN E

Evans and his coworkers in 1921–1922 first produced satisfactory evidence that reproduction is affected by a fat-soluble factor different from vitamins A and D. Using Osborne and Mendel's basal diet for rats (casein 18; cornstarch 54; lard 15; butter fat 9; salt mixture 4; plus 0.4–0.5 grams of dried yeast daily), they found that female rats put on this diet appeared in every way normal, had normal estrus, normal ovulation, normal implantation of eggs after fertilization but failed to produce normal litters. The expected young were resorbed instead of being born.

Supplementing with vitamins A, B, and D did not prevent fetal death but certain foods proved protective, viz., lettuce, whole wheat, wheat germ, and dry cereals. Consequently in 1922, Evans and Bishop postulated the existence of a new vitamin, calling it for the time being, substance "X". In 1923, they reported that its lack not only produced fetal death in female rats but complete sterility in males.

The existence of this vitamin was further confirmed by Sure who suggested that it be designated Vitamin E.

The early history of the vitamin has been reviewed by Evans and more recently summarized by Mattill who, with his collaborators, was a major investigator in developing the properties of this vitamin.

ANTIOXIDANT PROPERTIES

The chemical nature and properties of the tocopherols, the name now given to forms of this vitamin, are given in Chapter II, Figure 18.

The American Medical Association's Council on Foods in 1936 allowed no claims of value of vitamin E in human nutrition, holding that:

> "There are at present no adequate scientific data establishing the role of vitamin E in human dietetics"

It is included in vitamin tablets and capsules mainly for its ability to protect other vitamins from oxidative destruction; for its *antioxidant* value.

In 1931 Olcott and Mattill first showed that the antioxidant action of lettuce oil was related to its vitamin E content, later that this was also true of wheat germ oil. They also showed that the E content of experimental rations could be destroyed by rancid fat. Ferric chloride has also been used for this purpose.

In 1934 Olcott and Mattill prepared a concentrate of vitamin E from

wheat germ oil which proved to have both the biologic activity of vitamin E and antioxygenic potency. Acetylation did not destroy the biologic activity but did destroy the antioxidant action. From this fact and from collateral experiments they drew the conclusion that the antioxidant action of vitamin E depends upon the presence in the compound of hydroxyl groups. Later they demonstrated that the vitamin contains such a hydroxyl group or groups.

This evidence of antioxidant action and association with hydroxyl groups was produced before the vitamin was actually isolated and chemically identified. Hickman has suggested that perhaps by its antioxidant action, vitamin E has a place in the system as a control over the positive oxidation operations of vitamins such as those of the B complex.

TABLE XXVIII

Distribution of Tocopherol in Oils

(After Karrer)

OILS	NON-SAP FRACTION	ACETYL NUMBER
	per cent	
Wheat germ	13.4	14.4
Maize germ .	10 2	11.0-11.5
Lettuce . .	4.3	
Linseed ...	2.34	4.0
Olive.	0 935	10.6
Sesame	0 63	
Palm	0 55	1.9-8 4

RELATION TO MUSCULAR DYSTROPHY

In Mattill's review of the properties of vitamin E in 1938, he wrote:

"Equally mysterious is the paralysis in suckling rats from vitamin E deficient mothers. First observed and studied by Evans, its incurable nature and its prevention by wheat germ oil and vitamin E concentrates has been confirmed repeatedly. This condition was originally thought to be of nervous origin but it has now been definitely associated with skeletal muscles. The muscles exhibit extensive degeneration and their microscopic changes are not to be distinguished from those of *muscular dystrophy* in herbivora."

This is the muscular dystrophy previously observed and described by Goetsch and Pappenheimer.

The discussion of the relation of vitamin E to *muscular dystrophy* is detailed in Chapter XXIII. Correction or prevention of this condition is another possible function of vitamin E and may give it more importance in human dietetics than its biological activity on the reproductive system.

Its relation to prevention of habitual abortion is also reviewed in Chapter XXIII.

RELATION OF CHEMICAL PROPERTIES TO ASSAY METHODS

A colorimetric method was devised by Emmerie and Engel based on destruction of vitamin E by ferric chloride with reduction of the iron; the extent of whose reduction can be measured by the alpha-di-pyridil reagent.

Furter and Meyer claim that when alpha-tocopherol is treated with concentrated HNO_3 in absolute alcohol with short heating, an intensely red colored substance (probably an oxonium salt by addition of the HNO_3 to the tocopherolquinone formed by oxidative cleavage) develops and makes possible the estimation of tocopherol content by photometer. They claim this color is specific for tocopherol and has a maximum absorption band at 467 $\mu\mu$.

Karrer et al. report that it is possible to estimate tocopherol potency by potentiometric titration with gold chloride—2 mols. $AuCl_3$ equivalent to 3 mols. of beta-tocopherol. Using this method they report the values for oils given in Table XXVIII. In Table XXVIII are also given the acetyl numbers of certain of the oils. Mattill claimed that since the acetyl number indicates the measure of the hydroxyl groups it might serve as an approximate estimation of relative vitamin E strength.

CHAPTER XIV

NATURE AND FUNCTION OF VITAMIN K (MENADIONE)

In 1931, McFarlane and associates in Canada noted that chicks fed on ether extracted fish meal suffered a high mortality and extensive bleeding from small wounds. They also noted that the blood of these chicks failed to clot, even after hours of standing.

In the years just preceding Dam (1929–30) while studying fat metabolism in Copenhagen, noted marked hemorrhages and stomach lesions in chicks on a special diet. In 1935 Dam characterized this condition as due to deficiency of a "koagulation vitamin" or vitamin K; present in leafy vegetables such as alfalfa and in hog liver fat.

The pure vitamin was first isolated from alfalfa by McKee, Binkley, MacCorquodale, Thayer, and Doisy in 1939; and by Dam and Karrer in the same year.

The formation or synthesis of vitamin K by bacteria was demonstrated by Almquist and Stokstad in 1935. In 1939 McKee et al. isolated the K formed by bacteria in putrefied fish meal.

The chemical structure of these natural products and of other products having similar activity is given in Fig. 19.

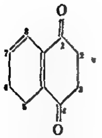
Animals with K deficiency show a remarkable tendency to bleed profusely as the result of minor injuries and blood drawn from such animals does not clot, but remains fluid for hours. There are various theories of blood clotting but general agreement that the process involves these two steps:

Step 1. Prothrombin *plus* thromboplastin *plus* ionized calcium unite to form the enzyme *thrombin*

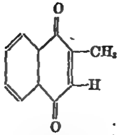
Step 2. Fibrinogen *plus* thrombin forms the clotted fibrin.

In brief, the conversion of serum-soluble fibrinogen into the solid fibrin requires the action of the enzyme thrombin. This enzyme does not exist in circulating blood but is formed from a precursor (prothrombin) by combination with a phospholipid of the blood platelets (thromboplastin or cephalin) and ionized calcium.

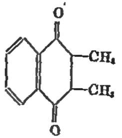
According to this theory it is evident that failure of blood to clot can result from insufficiency of one or more of the factors listed in Step 1. But there is definite evidence that in the phenomena observed by Dam and McFarlane the factor deficient in the clotting system is prothrombin and that for the manufacture of prothrombin, vitamin K is essential.



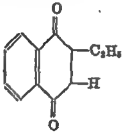
α-naphthoquinone
inactive



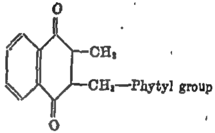
Active
CH_3 in 2
position
necessary



Much less
active



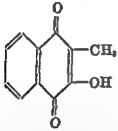
Still less
active



Active
K_1
Any other change
reduces activity

Structural changes affecting K activity

Almquist & Klose showed phthiocol effective

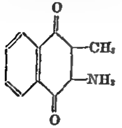


Phthiocol first isolated by
Anderson & Newman from alkaline
hydrolysate of the lipids of *M. tuberculosis*

1/500th the activity of K_1
Perhaps a derivative of K_1

Water soluble combinations of quinones with dibasic or tribasic acids have some activity.

Amino naphthols also active



As active as menadione

Used as water soluble hydrochloride salts

K_1 not toxic in large amounts.

FIG. 31

In 1938 Quick devised a method of measuring the prothrombin concentration of the blood which is today widely used to diagnose the existence of vitamin K deficiency. The essential feature of the Quick test is that it eliminates two factors of Step 1 by adding thromboplastin and calcium in excess. The clotting time becomes a direct measure of prothrombin deficiency.

VITAMIN K NOT A SINGLE SUBSTANCE

As shown in Chapter II, the forms of vitamin K (see Fig. 19) isolated from alfalfa (K_1) and from fish meal (K_2) are different in composition; K_1 having a smaller molecular weight than K_2; and both K_1 and K_2 have a larger molecule than the naphthoquinone product now produced in the laboratory and called "*Menadione*."

The term Vitamin K therefore is inclusive of a group of products, all derivatives of the naphthoquinone nucleus.

The League of Nations Commission has suggested that the unit of K be taken as the effect of one microgram of pure Menadione (2-methyl-1,4-naphthoquinone).

FUNCTIONS OF VITAMIN K

It is recognized today that human beings have two possible sources of vitamin K, K supplied by the diet and that synthesized by intestinal bacteria. Rats, dogs and man readily absorb the bacteria formed product and are less apt to show K deficiency on that account. Fowl appear unable to synthesize their needs and hence readily develop K deficiency when the diet lacks this factor.

As stated above, the principal function of the vitamins K appears to be the control of prothrombin formation. The site of this formation seems to be the liver. This is evidenced by the fact that liver disease is invariably accompanied by drop in prothrombin concentration and the fact that in animals with excised livers, the feeding of vitamin K fails to restore the prothrombin to the blood.

We still lack an explanation of how the vitamin forms prothrombin. A methyl group in position 2 of the naphthoquinone nucleus appears essential to its action. For other changes in structure affecting activity, see Figure 31. Vitamin K is not a part of the prothrombin molecule.

To be effective the vitamin must reach the liver. The compounds that occur in nature all are insoluble in water. They are soluble in fats. There are now a few synthetic K analogues that are water-soluble and these are of value for intravenous injection. The failure of ingested vitamin may be due to absence of bile salts which aid its absorption and utilization. It may be due to damage to the mucosa of the intestinal tract preventing passage into the blood. And, finally, damage to the liver can prevent prothrombin formation.

PART II

THE AVITAMINOSES

CHAPTER XV

VITAMINS AND DISEASE

There is nothing new in the conception that inadequate diets cause disease. Even primitive peoples recognize this relationship and provide special foods to prevent and cure certain diseases. Price's experience would indeed suggest that in some respects the lore of primitive man is superior to our knowledge for it insures better dental development and physique and provides more effectively for the nutritional requirements of pregnancy and puberty. But the dietary prescriptions of natives are strictly empirical, rich in both fallacy and truth.

In our early history are many illuminating comments on deficiency diseases. In calling our dogs Toby we keep alive the story of that blind Tobiah who was guided by his dog until an Archangel prescribed fish liver for his xeropthalmia whereupon the skin fell from his eyes. Early physicians knew something of these diseases. Beriberi was described in the 7th century, scurvy in the 13th and pellagra in the 18th. The curative powers of certain foods became evident many years ago. Lind's successes in treating scurvy are two hundred years old and the beneficial effect of cod liver oil in rickets was generally recognized twenty years before vitamin D was discovered. Perhaps the most prophetic observation of all is that of Budd, who more than a hundred years ago wrote that scurvy was due to the "lack of an essential element which it is hardly too sanguine to state will be discovered by organic chemistry or the experiments of physiologists in a not too distant future."

Many clues to the entire field of the avitaminoses may be found in the literature of the years that followed. These were translated into facts as a direct result of the studies of Liebig and Lavoisier which made inevitable an investigation of the qualitative aspects of nutrition. A clear conception of the subject and its importance and the groundwork for its methods of study were laid by two outstanding chemists, Funk and Hopkins. Funk, in 1911, extracted from rice polishings a few milligrams of a crystalline substance which cured experimental beriberi and coined the name "vitamine." He also predicted with remarkable accuracy which diseases would prove to be due to "vitamine" deficiency. Hopkins, in the years from 1906 to 1912 established the groundwork of our technique and foresaw the importance of the accessory food substances and the future course of developments.

Thus the idea that diets deficient in certain elements may result in

disease is hardly new. But two conceptions have grown out of the work of the past twenty years which are either new or are given new significance by recent developments. They are the conception of the *sub-clinical deficiency* and the public health implications of nutrition.

The conception of sub-clinical deficiency was an inevitable result of the basic vitamin technique, the feeding of a diet graded in its deficiency. For in any experiment of this kind one is bound to reach levels of intake where the question arises, "Is that all that this particular animal requires?" Some years ago in measuring the rate of growth of the incisor teeth of guinea pigs vitamin C was supplied as orange juice. The end point became a problem in judgment for as the ascorbic acid was increased additional vitamin A was also supplied. And the question arose whether maximum growth is physiologically desirable.

From interest in marginal deficiencies in experimental animals attention naturally turned to man and slowly means were developed to measure and identify human malnutrition of sub-clinical degree. Unfortunately none of these criteria is satisfactory in the sense that the demonstration of *Mycobacterium tuberculosis* in the sputum is diagnostic of tuberculosis. One of the few which are widely accepted at present is the blood plasma concentration of ascorbic acid. Yet we have no proof that values less than 0.6 mgm. per 100 ml. signify disease. Indeed there is much evidence that such values are frequently associated with excellent health. It is indeed rather characteristic for a person with a low blood ascorbic acid level to revert to the same value once intensive therapy ceases. The concentration appears to be a relatively fixed value for the individual.

Radiographs of the wrist bones are our best criterion of rickets but their interpretation is influenced by the standards of the examiner. The diagnosis in marginal cases is subjective. Fifteen years ago, when every orthopedic clinic in New York City was treating gross rachitic deformities, Hess estimated the incidence of rickets at 75 per cent. Higher estimates are frequently made today yet clinical rickets of the kind formerly seen has become rare.

Each new proposed test has progressed through stages of enthusiasm quickly followed by doubts of its specificity and finally realization that interpretation is difficult, subject to many errors and the results of doubtful significance. It is uncertain that a single test of this kind is diagnostic unless evidence is present showing response to specific therapy. Yet nutritionists have lately discovered that few if any of these lesions respond promptly to treatment. Like the blood ascorbic acid they become individual characteristics.

We lack satisfactory criteria of sub-clinical deficiencies. What do our morbidity and mortality records tell of the prevalence of nutritional

failures? They show that clinical deficiency diseases are uncommon. Clendenning polled six excellent hospitals in widely separated parts of the United States and found the incidence to vary between 0.13 and 0.4 per hundred admissions. Jolliffe, McLester and Sherman report that of 35,813 Bellevue Hospital patients 197 or 0.55 per hundred were recognized as having a dietary deficiency. By including cases of hypochromic anemia and of "malnutrition" they raised the estimated incidence to 1.75 per hundred. Even this rate, which includes a third *suspected* of deficiency (the "malnutrition" cases) and a third due to anemia in which dietary deficiency is one of the least common causes, is but a fraction of the estimated incidence among the population at large.

Actually the discrepancy is much greater than the figures suggest for *few hospital cases are primarily due to inadequate diets.* Jolliffe emphasizes this in a discussion of "conditioned malnutrition" by which is meant deficiency secondary to other diseases. The choice of the term "conditioned" indicates that such deficiencies are refractory to treatment by dietary means unless the predisposing cause is corrected. For the present discussion, concerned with causation, it is better to refer to such cases as secondary deficiency disease. Their importance is emphasized throughout the following chapters. There appears to be no disagreement that they represent the bulk of the avitaminoses seen in hospitals. Thus in a recent report by Spies covering 278 cases of multiple deficiencies it is said that 148 followed organic disease, 115 were secondary to alcoholism and 6 per cent were presumably due to diet alone.

Nutritionists criticize the validity of hospital records on the ground that many cases are not recognized and because of "mislabelling in the traditional 'precedence' given to certain diseases over others." Doubtless both are true charges. Physicians probably often do not recognize nutritional stigmata or record them in summarizing their cases because their attention is directed to more urgent, painful or threatening diseases than those due to sub-clinical deficiencies. The physician confronted by the diagnostic problems of early cancer of the lung, the handling of rheumatic fever, training a diabetic to control his diet and insulin or occupied by the care of lobar pneumonia will naturally be less attentive to flecks in the conjunctiva or vascularization of the margin of the cornea. Other diseases which have considerable public health importance, for example trichinosis, are known to escape detection for similar reasons, precise and reliable tests are lacking and most illnesses caused by *Trichinella spiralis* are minor.

The same is doubtless true of mortality records. Yet the question posed by an official committee, "How much malnutrition is concealed in the 370,000 deaths recorded in 1938 under the heading of the diseases of the circulatory system" seems to be misleading. Adequate samplings of such

deaths are annually studied post mortem and the causes of heart disease are well known. Unless arteriosclerosis, rheumatic fever, syphilis and congenital lesions are to be ascribed to sub-clinical deficiency we know that *very few* cardiovascular deaths in the United States are due to malnutrition. In more than 5000 necropsies at Grasslands Hospital during a period when a lively interest in deficiency lesions has been present but five frank cases of death due to an avitaminosis have been recognized. Isolated lesions have been much more frequent. It would seem that in the matter of emphasis physicians have taken the only justified course.

Physicians doubtless hold more reservations regarding the significance of vitamins than do nutritionists. Medicine is old and nutrition is relatively new. Medicine teaches that comparable developments usually become more complex than is at first suspected. The growing realization that most cases of vitamin deficiency are secondary to other diseases reinforces this opinion, and there are doubtless other qualifying factors. We may well consider the possibility of *adaptation* to poor diets, the obverse of the abnormal susceptibility of sick people to vitamin deficiency. Adaptation was first suggested to us by Aschoff who had noted that troops home on furlough were more susceptible to scurvy than civilians. Aschoff investigated the phenomenon and concluded that the civilians were able to *adapt* to the deficiency because their diets had been slowly restricted over a long period of time. Kellogg has recently written: "Over a long evolutionary period, both people and animals have some ability to adjust themselves to deficiencies in the diet. Groups of people that have lived for many generations on calcium-and-phosphorous-deficient soils seem to be more efficient in the use of these elements than others. The teeth of Europeans will become diseased, for example, in tropical areas where the natives have good teeth."

A recent report by Spicknall, Fishburn and Baum of the United States Public Health Service on the diets in German internment camps includes the following statement. "It was observed . . . that the Americans tolerated the diet less well than the Germans did, probably because the Americans had been placed abruptly on a reduced ration, while that of the Germans had been scaled down gradually." And van Veen, a skilled and experienced nutritionist, has found it necessary to call attention to the same possibility. "Yet numerous investigations into the diets of many regions of the tropics and the Far East have proved that these diets do not always induce a state of malnutrition and ill-health. This leads us to wonder whether the customary (Western) standards and requirements are not sometimes too high, or whether they are applicable to all races and climates. This query applies to the so-called 'optimal' as well as to the 'minimum adequate' requirements which, no matter how difficult to define and to determine, are easily understood."

A further example of the type of evidence which tempers the enthusiasm of physicians is implicit in the current studies of heightened resistance to poliomyelitis of thiamine deficient mice. The suggestion was made by Foster that the virus of poliomyelitis may be more exacting in its nutritional requirements than its host. The implications are obvious and important from a medical point of view and can be correlated with much other experience including the para-aminobenzoic acid requirements of organisms referred to in Chapter XX and the mutually exclusive nature of certain infectious diseases commonly spoken of as *interferences* which we (GD) have suggested may be due to competition for specific nutrients. The term interference has also been used to designate those instances in which a vitamin deficiency is precipitated by a destructive agent in the food (see Chastek paralysis) or related means; biotin deficiency by avidin, rickets in chickens by sulphur (Holmes, Deobold and Herrick).

It seems odd that deficiency diseases have increased over recent years when people had more of what seemed to be the right kind of food. Phipard writes: "Such shifts in food consumption as have taken place between 1936 and early 1942 are in the direction of improving the Nation's diets." Wells has compared the estimated average per capita consumption of various foods in the United States in the period 1909–1941. During that period the average consumption of certain protective foods has increased greatly.

	Increase (%)
Citrus fruits	200
Evaporated milk, cheese, ice cream	145
Canned fruits and vegetables	135
Fresh vegetables	35
Fluid milk	10

There have been other reforms which must have had considerable beneficial effect: canning methods improved to preserve the vitamins; the popularization of milk as a beverage; and the enrichment of white flour to name the more important.

If despite these improvements our diets are still grossly inadequate one might well ask with Carlson, "How do you suppose our ancestors carried on, in the total absence of modern knowledge of food chemistry, vitamin requirements and the alleged necessity of 'a pint of milk a day'? I do not think the Sioux Indians got much milk from the wild buffalo." Other investigators have been troubled by this inconsistency and by the physical stature and prowess of people who subsisted on diets that the modern American would regard as a short cut to the grave.

These remarks would be misleading if the reader assumed from them that the authors subscribe to the view that the diagnosis and treatment of

all manifestations of vitamin deficiency are either fruitless or that malnutrition is not an extremely important public health problem.

The recognition, study and treatment of the manifestations of vitamin deficiency have already led to clear-cut and practical results in the treatment of pellagra, certain forms of neuritis, the control of hemorrhage during icterus, improved methods of rickets prevention and the successful treatment of certain infrequent but formerly obscure nervous disorders. The promise in the future is bright especially in the field of hepatic cirrhosis. The discovery of a preventive for cirrhosis would rank second only to the demonstration that nicotinic acid is curative and preventive of pellagra.

Great advances have been made toward a better understanding of physiology and pathology. Moreover they promise to facilitate the investigation and control of many unrelated diseases. Reference to examples of this kind will be found in the succeeding chapters.

And finally there is sound evidence that the accomplishments of nutrition are very significant. Though we cannot measure these conditions sufficiently well in adults to determine what changes in diet will accomplish, the growth records and physical improvement of our children show great benefits to the young. It is reasonable to assume that equally valuable benefits can be secured for their parents even if they cannot yet be measured. The known facts of animal nutrition suggest that this is true. Perhaps measurements of physical endurance or work output will afford us a yardstick. Barborka, Foltz and Ivy have collected evidence of the physical *inefficiency* of volunteers on thiamine low diets and unpublished experiments from certain war factories support these conclusions.

The problem is doubtless much more complex than was formerly suspected but hardly less important. We agree with Sebrell that nutrition is "perhaps the greatest and most complex problem in preventive medicine that this country has ever had." Not only is it complex because human nutrition is complex but also because crop production, soil enrichment, distribution, food processing, preparation, education and basic economic improvements are all essential to a satisfactory solution.

If animal experiments are a guide, and there is no reason to doubt that they are, nutrition will bring a measurable lengthening in the period of "full adult capacity" as well as the increased stature, rate and sturdiness of growth which we have noted in the young.

There is apparently good reason to believe that the differences between average and optimal diets may be indirectly measured in pregnant women. It is of course reasonable to assume that the demands of pregnancy and the sensitivity of the fetus to maternal deficiencies make pregnancy a very acute criterion of dietary defects. Ebbs, Tisdall and Scott observed 400 women throughout pregnancy. One group had a poor diet, a second group

had a poor diet plus supplements and the third group had a "fair" diet and had been instructed in the selection and preparation of their food. The last two groups excelled the first in freedom from complications, number of miscarriages, still births and premature infants. Furthermore the infants had less sickness and fewer died.

These observations are confirmed by Burke, Beal, Kirkwood and Stuart. In the cases they observed mothers whose diets were grossly inadequate gave birth to every still-born infant, every infant but one who died shortly after birth, the majority of infants with congenital defects and all premature and functionally immature infants. The association between the health of the infant and the mothers diet is shown in figure 32. It seems

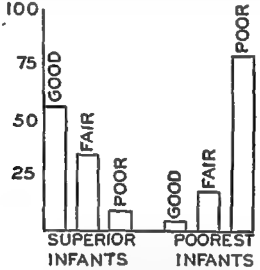


Fig. 32. The relationship between maternal nutrition and the physical condition of the infant Based on the studies of Burke, Beal, Kirkwood and Stuart. The percentage of "superior" and "poorest" infants having mothers whose diets were rated "good," "fair" or "poor "

reasonable to assume that if such differences can be demonstrated by the provocative test of pregnancy similar if less marked effects occur at other times.

It may be noteworthy that as a general rule the clearest demonstrations of improved physique have followed improvements in the regular *diet* rather than the administration of purified supplements. This may be due to the advantages of nutritional congeries or to x factors yet undiscovered. Or perhaps we have overemphasized the vitamins, as Hoelzel suggests. That x factors must still be accounted for is evident from recent studies by Elvehjem in small animals, the work of Smith, Curry and Hawfield and Lambooy and Nasset with dogs, the studies of Waisman et al. with mon-

keys, and many others. Foster, Jones, Henle and Dorfman demonstrated that synthetic diets containing all known vitamins are inadequate by showing a higher mortality and stunted growth in the young, a study comparable to the human experiments just described.

Whatever the ultimate solution of these problems and the means devised for estimating the adequacy of a diet a review of the evidence encourages the belief that man has acquired a new tool of great effectiveness for the improvement of his physical structure. The newer nutrition has indeed challenged certain opinions regarding the fixity of the human inheritance, the expectancy of life and the estimates of normal energy and vitality. These hopes for the future are well described by Sherman to whose "The Science of Nutrition" the reader is respectfully referred.

CHAPTER XVI

VITAMIN A DEFICIENCY

Only in the Far East is vitamin A deficiency an important primary cause of disease. Elsewhere deficiency is usually secondary and of minor medical significance.

Vitamin A is of great *nutritional* importance as a *growth factor*, the characteristic which led to its recognition, and because intakes considerably greater than maintenance doses confer benefits in health and longevity.

VITAMIN A REQUIREMENTS

Although vitamin A has been studied for thirty years the postulated human requirement is still largely empirical. This is true because depletion develops irregularly and slowly (probably because of liver stores), because of lack of agreement on the significance of the criteria of early deficiency and finally because various widely distributed carotenoid pigments serve as vitamers. Variations in the structure of these provitamins and the efficiency of their conversion makes human vitamin A nutrition a very complex affair.

The Committee on Food and Nutrition of the National Research Council has recommended the following vitamin A intakes:

Infants up to one year of age	1500 Int. Units
To age seven	2000 Int. Units
To age ten	3500 Int. Units
To age thirteen	4500 Int. Units
Adults	5000–6000 Int. Units
During lactation	8000 Int. Units

These values are probably about three times as much as is needed to maintain normal dark adaptation (Wagner, Guilbert, Howard and Hart). There seems to be excellent justification for that multiplier in Batchelder's experiments in which rats fed throughout their lifetimes on diets containing the minimal adequate amounts of vitamin A and two, four and eight times as much showed increased benefits from the two and fourfold increments.

The additional allowance for lactation is calculated to replace vitamin lost in mammary secretion.

Vitamin A requirements are easily and cheaply met if vegetable and dairy products are abundant. In infancy the practice of giving cod liver oil as a rickets preventive automatically supplies large amounts of vitamin A (the exact amount varies with the preparation and source of the oil)

and a quart of milk provides all the vitamin A needed by children twelve months old.

It is encouraging to note in a recent analysis by Stiebeling that these levels seem to have been met in the United States during the period 1936–1940 and were nearly met in the period 1920–1924 although the records referred to apply to our gross food supply and do not allow for uneven distribution. von Drigalski estimated the German consumption to be 3 to 5 mgm. carotene for rural groups and 2 to 3 mgm. for city dwellers. The latter is the equivalent of approximately half the average American consumption.

FACTORS INFLUENCING VITAMIN A REQUIREMENTS

Bio-assays of a given preparation of Vitamin A yield different results in different laboratories and at different times. The effort to standardize vitamin A unitage by spectroscopic tests has failed. The results of bio-assay are not reproducible but they do estimate the characteristic biologists are primarily interested in.

The discovery that vitamin E, a natural constituent of fish liver oils, modifies the absorption and vitamin A effect of natural vitamin A preparations appears to be an important element in the problem. Originally demonstrated by Moore it has now been confirmed in various ways; by demonstrating greater liver storage of vitamin A if vitamin E is included in the diet and by using the standard growth curve in experiments in which the vitamin A intake is fixed but the E supplements are varied (Hickman, Harris and Woodside). The role of vitamin E has not been clearly defined at this time. Moore's original explanation was that it might stabilize vitamin A solutions as other hydroxy aromatic substances are known to do.

Conversely several substances have been shown to adversely affect vitamin A in the gastro-intestinal tract. Sherman showed that linolates and linolenates did so and palm oil and rancid and stale diets have been found even more destructive.

The *condition* of the food modifies the intestinal absorption of vitamin A. Mention has been made of the adverse effect of pancreatic dysfunction which impedes the hydrolysis of the vitamin esters and absorption. Caro-

of its carotene as liver vitamin A, while cooked carrots yielded 30 per cent. The difference was less in the case of spinach.

The liver plays a dominant role in vitamin A metabolism. Moore found the liver to contain 100,000 times as much vitamin as all other fat depots. The vitamin is present in both the parenchymal and Kupffer cells which

re the last to surrender and the first to store vitamin A. There is indirect vidence that the Kupffer cells play a part in the conversion of carotene o vitamin A. This is based on RE blockade experiments. There is ittle or no vitamin in organs other than the liver, kidneys, adrenals and onads. It appears at the site of formation of the sterone hormones; the drenal cortex, the interstitial cells of the testis and the ovary (Ragins nd Popper). No functional relationship was claimed. Vitamin A dis-ppears from the ovary during pregnancy. The conversion and storage f vitamin A are subject to forces which are still unrecognised. Thus Moore found that the efficiency of carotene conversion varies greatly with he level of vitamin A storage. Deficient animals converted most carotene o vitamin A whereas saturated animals converted little. The excess caro-ene was excreted in the feces.

The relationship between maternal and foetal vitamin A has been recently tudied by Lund and Kimble who found plasma vitamin A to vary as the lietary intake, but to be apparently unrelated to the plasma carotene oncentration. The slowly falling levels of plasma vitamin A during the 2nd and 3d trimesters of pregnancy suggested to them that an additional 5000 Int. Units. should be provided during the 2nd trimester and 10,000 luring the third. Lund and Kimble also demonstrated an abrupt and pronounced rise in plasma vitamin A during the first 24 hours postpartum. In some women the rise occurred within a few hours following emptying of the uterus (see Fig. 33).

These workers confirmed the opinion that the infant is relatively poor n vitamin A (plasma values average half that of the normal mother). During maternal depletion both mother and infant exhibit a strong tend-ency to husband vitamin A. An extremely interesting observation made luring the course of these studies is the constitutional difference between ndividuals. Thus Lund and Kimble found wide variations in the plasma values in newborn twins.

Human milk contains from 5 to 10 times as much vitamin A as does cow's milk, and colostrum is twice as rich as milk. These facts imply that the infant's requirements are large during the first months of life and should guide physicians in prescribing for infants when mother's milk is unavailable.

The various carotenes yield quite different amounts of vitamin A. Beta-carotene, the most active, was long thought to supply two molecules of vitamin A because of its formula. It is now known that this is not true and if all other factors were disregarded its vitamin A value would still be but 50 per cent on a weight basis. Yet it yields twice the vitamin A that alpha-carotene does. Of other dietary factors which alter the absorp-

ins, we find that "it has been oftenest observed as an acute epidemic fection attacking large numbers of people living under nearly identical bnormal conditions." "Older writers have said it especially affects persons ith pigmented eyes." (We now know that pigmentation of the eyes is nother expression of A deficiency.) "Impaired nutrition and strong sunght have almost always been associated with its occurrence." (The effect f light on visual purple has been explained.) "Dryness of the scleral onjunctiva with formation of scaly patches—conjunctival hyperemia with achrymation and photophobia—are frequently associated."

In Hippocrates' time, too, they had discovered a treatment whose basis s understandable only since the discovery of vitamin A. To them it was urely empirical. An ointment was made from the juices exuding from oasting liver and applied to the eyes, or they were steamed with vapor rom the water in which liver was cooked; to which was added the recomendation to eat the liver. Cod liver oil was also highly commended.

It may be seen from the foregoing that long before the discovery of itamin A physicians were familiar with the major features of its deficiency ffect and had empirically developed some sound measures of treatment.

The earliest lesion attributed to vitamin A deficiency was xerophthalmia hich occurs in rats as well as man. This discovery explained the early mpiric treatment of cases of xerophthalmia and the clinical observations f associated night blindness were promptly ascribed to the action of the itamin as well. The identification of characteristic skin lesions came elatively late and was only established by the studies of Frazier and Hu. This no doubt was due to the irregularity with which skin lesions occur in uman cases and their absence in the usual experimental animal, the rat.

Less interest in prevention seems to gave existed in more modern times nd the disease has persisted in many places, and has often occurred in najor epidemics. Russia, Brazil, Denmark, and the Mediterranean lands, ave all had epidemics of hemeralopia and xerophthalmia, and cases are till common in China and India.

In Tientsin six per cent of the patients attending an eye clinic in 1929–930 had conjunctival xerosis. Nicholls gives the incidence of the disease n Indian institutions he had inspected as follows:

	per cent
Charity boarding schools	[illegible]
Poor vernacular schools	29
Upper class schools	3
Mental asylums	44
Mental asylums (on European diet)	2

The disease is also common in Yucatan and in Labrador. Pillat says hat the poorer Chinese are almost permanently on the border line of itamin A deficiency.

studied the vitamin A absorption in cases of celiac disease. A large test dose of vitamin A was given by mouth and the blood vitamin A determined at 2, 4, 6, 9, 12 and 24 hours. Normal absorption is sufficiently rapid to produce a peak concentration within 4 hours. In 10 tested cases of celiac disease absorption was much reduced and delayed.

Diabetes mellitus is often associated with hypercarotenemia which is thought to be due to an inability of the liver to convert carotene to vitamin A. Thus Ralli and her associates have demonstrated that diabetics not only have higher blood concentrations of this pigment but that they cannot handle excessive amounts as effectively as non-diabetics do. Brazer and Curtis examined juvenile diabetics by means of the Biophotometer, testing the influence of both carotene and vitamin A on their adaptation. They found evidence of poor adaptation to be very common and several of their patients gave histories of night blindness as well. Carotene did not correct this defect but vitamin A did indicating that conversion was abnormal. Thus the diabetic may become deficient in vitamin A because he is unable to convert provitamin A into the active form.

THE PATHOLOGIC ANATOMY OF VITAMIN A DEFICIENCY

The pathologic anatomy of vitamin A deficiency is essentially the same in experimental animals and in man. However, the experimental evidence is more complete and orderly, and will be considered first.

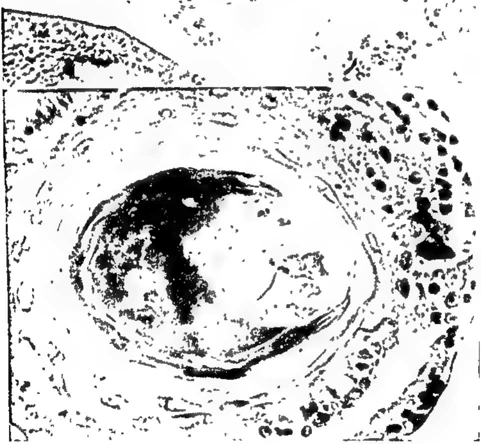
Epithelial Metaplasia

The specific anatomic effect in the rat, guinea pig, monkey, chicken and other animals, is the loss of ability to maintain various specialized epithelial surfaces. The result is the replacement of such surfaces by less specialized cells, a change which has been consistently found since Mori first observed the lesions in the eye. They may be regarded as specific lesions of this particular deficiency, and may be prevented or reversed in the rat by amounts of the vitamin estimated to represent but $\frac{1}{1,000,000}$ of the body weight. Furthermore, the lesions may be produced by degrees of deficiency which do not limit growth, formerly considered a cardinal sign of vitamin A deficiency.

The lesions commence simultaneously in various foci. In the rat, a general order of development was pointed out by Wolbach and Howe: first the respiratory mucosa, then, in order, the salivary glands, eyes, glands of the gastro-intestinal tract, parocular glands, and pancreas. However, exceptions to this rule occur in the rat and among various species. Lesions of the eyes were not found in the monkey—Tilden and Miller—or guinea pig—Wolbach and Howe.

The complete picture of vitamin A deficiency in the rat and guinea pig

Fig



Fig

PLATE I Experimental vitamin A deficiency The effect of deficiency on the endometrium of a rat Figure 1 shows the endometrial gland in the center of the photograph to have undergone metaplastic involution The gland is filled with keratinized epithelium Notice that the lesion is purely focal Figure 2 shows the same gland more highly magnified

Fig. 1

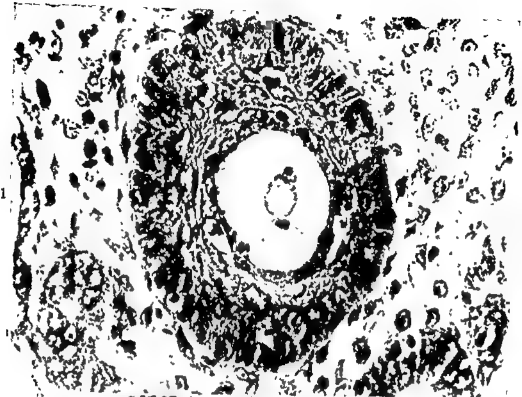


Fig. 2

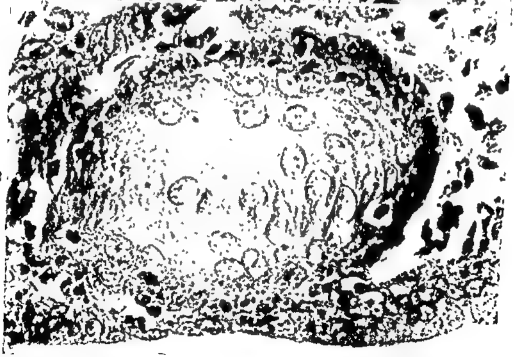


PLATE II Experimental vitamin A deficieny. Endometrial lesions in the rat. In both figures the deeper cells may be seen to have been very active Mitotic figures are numerous The excess cells have piled up within the glands In figure 1 the epithelium is stratified.

has been drawn by Wolbach and Howe. By the time the rat is retarded in growth, assumes a hunched position, and shows encrusted eyelids, and a rough coat, keratinized epithelium may be found in the nares, larynx, trachea, bronchi, in the submaxillary, parotid and accessory salivary glands, in the urinary bladder, ureters and kidney pelves, the uterus, oviducts, prostate, epididimis and seminal vesicles, the conjunctiva, cornea, lacrimal glands and the ducts of the Meibomian glands, and in the thymus, where the Hassall corpuscles are magnified and often transformed into cystic spaces filled with keratinized epithelium. Similar changes occur simultaneously in the enamel organ leading to extensive changes in the teeth.

In all of these sites but the enamel organ, the process is identical; and in that organ substantially so.

In the early stages of the deficiency, nests of darkly stained germinal epithelium may be seen to undergo rapid growth. As it grows, the secretory or duct epithelium overlying degenerates and sloughs off, and the foci grow to form islands of stratified squamous epithelium. These first extend laterally to undermine the adjacent surfaces and eventually, if the dietary defect is sufficiently prolonged and severe, extend to replace the entire surface of the affected organ. With lesser deficiency, alternating areas of normal and replacement epithelium occur side by side, and the former remains normal in appearance.

The process begins distant from other stratified epithelium and quite independent of it. According to Seifried the most important vitamin A deficiency in domestic animals occurs in chickens where it causes serious economic losses and is usually confused with common infectious diseases, chicken pox, coryza contagiosa and infectious bronchitis. Symptoms appear 40 to 60 days after inauguration of deficient diet and death occurs a month later. The nasal passages and sinuses are filled with masses of clear mucoid material which later becomes opaque and caseous. If it is removed the membranes beneath are found to be dry and opaque. In the larynx and trachea thick patches of caseous material collect in the mucous glands and crypts and the mucosa is likewise metaplastic and dry. Seifried closely followed the histological sequences. These start with atrophy, loss of cilia, shrunken nuclei and loosening of masses of epithelium. New cells form beneath, often in syncytial masses and grow to form areas of flattened squamous epithelium. Keratohyalin granules may be found in the cells. Balloon giant cells occur and mucous secretion, of course, ceases. Particular attention was paid to phenomena which occur in the nuclei of degenerating cells and which often end in the extrusion of nucleoli which then resemble Guarnieri bodies. The lesions in the mouth resemble those of fowl pox. The submaxillary glands are affected early in the disease and, with occlusion of the ducts, often become distended and cystic.

Some disagreement exists as to whether the metaplasia should be regarded as a true or replacement metaplasia. Our opinion is that the latter is *the more probable* explanation because of the evidence which shows epithelial deterioration preceding metaplasia. This consists of the opinion that in the trachea the ciliated epithelium shows loss of function before structural changes occur; the evidence of Pillat that in the corneal epithelium serious damage occurs in the mitochondrial apparatus—which contains much lipoid—and in the Golgi structures before frank lesions appear; and the discovery that the purine content of tissues are reduced during deficiency. The purines are required in the formation of cell nuclei. To these reasons might be added the general observation that it is more harmonious with what is known of other vitamin deficiencies, to consider that a material essential to specialized cells is lacking in vitamin A starvation.

The primary consequence is the loss of function of the affected surface. In the case of the trachea, the loss of cilia precludes proper cleansing of that part, and in the conjunctiva the loss of mucous secreting cells has a similar adverse influence. Similar effects must occur elsewhere although they are less obvious. Two other major sequelae have been repeatedly emphasized.

The most striking is the blockage of gland ducts leading to stasis of secretions and, with the interruption in the intact surfaces, to infection. The frequency of infection in organs diseased as a result of vitamin A deficiency seems to have varied in different experiments, probably in part due to the degree of cleanliness of the cages and animals, and led earlier experimenters to consider that infection invariably occurred and predisposed to the metaplasia.

This is not the case, as Wolbach and Howe and others have shown. Proof is also afforded by the study of the lesions of the enamel organ, the uterus and oviducts where infection rarely occurs.

The associated abscesses and metaplastic changes have always been most common about the mouth and its glands where exposure to infection is obviously easy and, indeed, the rule. Nevertheless, complicating infection is very common. Indeed infectious lesions account for the death of many experimental animals if the closest attention is not given to prevent them. Orten, Burn and Smith report a mortality of 54 per cent in their experiment, described elsewhere, in which deficiency was maintained for exceedingly long periods. The causes of death were tracheal obstruction (mucous plug) otitis media and sinusitis.

Mellanby reported that examination of 92 rats on an A deficient diet showed that 44 per cent had infection of the genito-urinary tract including pyelonephritis, cystitis, renal and bladder calculi, hydronephrosis, dilated

Fig 1

Fig 2

PLATE III Fig. 1. The costo-chondral junction of a rat fed a diet deficient in vitamin A. The photograph shows cessation of cartilage growth as well as atrophy of osteoblasts. These changes result in failure to grow. What bone is present is dense and substantial. Fig. 2. A lower incisor from the same animal. The photograph shows the buccal surface of the pulp. Atrophy of the odontoblasts was even more pronounced on the lingual surface. The denticle formation (center of photograph) is regularly seen in protracted cases of vitamin A deficiency. Notice that the dentin is dense although irregular.

eters, and distended bladders; 20 per cent had otitis media; 20 per cent d acute inflammation of small or large intestine—acute enteritis involv-g duodenum and jejunum and hemorrhage at the pyloric end of the omach; 9 per cent had lung infections; 38 per cent had xerophthalmia; 2 per cent had abscesses in the floor of the mouth.

The other significant consequence of the epithelial changes lies in the terference with the functions of the glands and organs involved. Wol-ach considers these effects as sufficient to explain the anemia and cessation f growth. The lesions in the uterus and germinal cells produce sterility. completely obstructed kidney is occasionally seen. Urinary calculi are ommon.

Wolbach and Bessey emphasize a characteristic of the metaplasia of itamin A deficiency which may be unique. The cells of the stratum erminativum preserve the identity of the original epithelium. They can ssume the original morphology and function without undergoing division vitamin A is supplied. From this observation they infer that the nuclear hromatin is not affected by the deficiency.

Tooth Changes

The tooth lesions were recognized later than the epithelial changes. They are equally striking and characteristic. Wolbach and Howe consider hey are the most important dental effect of any of the deficiencies.

The deficiency expresses itself in atrophy and metaplasia of the enamel rgan. Enameloblasts become replaced by stratified squamous epithelium. s a result, there is loss of enamel and exposure of dentin which gives the eeth a chalky appearance. Simultaneously, the odontoblasts within the ooth atrophy and an irregular line of thin odontoblasts with irregular slands of denticles form. This occurs last in the labial surfaces so that he tooth has a thicker labial than lingual wall. Schour, Smith and Hoff-nan measured these differences by injecting a dye (alizarine Red S) which tained the proximal margin of the dentin. At various intervals thereafter eeth were examined and the accretions of dentin could be readily measured. Whereas 16 μ formed normally teeth of deficient animals showed as much s 19 μ on the labial surfaces and as little as 6 μ on the lingual surfaces of he incisors.

Additional information has also been contributed by Orten, Burn and Smith who managed to prolong deficiency by feeding small vitamin A upplements adjusted to each particular animal. Thus an incipient vita-nin A deficiency was maintained for periods up to 1 year. Growth was etarded but the growth curve had a normal configuration. The most triking and consistent finding was in the incisors which became opaque, listorted and twisted. Ridges appeared on the surfaces and in some cases

Urinary System

Higgins, McCarrison, Van Leersum and Steiner, Zuger and Kramer, to mention but some, have all *found renal calculi common in deficient animals.* The calculi are usually composed of calcium and tend to disappear if the diet is corrected. The opinion held by most investigators is that these are simply concretions formed about desquamated epithelium, a consequence of metaplasia of the kidney pelvis, ureters and bladder. Most of the studies have been made in rats which are prone to spontaneous calculi but Steiner, Zuger and Kramer were also able to produce calculi in [illegible] of 35 guinea pigs fed a vitamin A deficient diet. Mineral intake and pH of the urine were controlled in these experiments. Wolbach, however, believes *other factors than the vitamin are involved since calculi did not occur in* his animals. Erspamer searched for gall stones in deficient guinea pigs. Amorphous masses were found but nothing resembling calculi such as occur in man. (Many of the renal calculi which have been described seem to have been but inferior imitations of those which occur in man.) No success has followed attempts to treat renal calculi in humans with vitamin A. However cases may well occur, but rarely, in which deficiency and kidney pelvis metaplasia may incite the deposition of a urinary stone. Metaplastic lesions are not rare in human material and if the character of the urine *is suitable should predispose to stone formation. That this* mechanism is responsible for other than exceptional cases seems unlikely.

Lesions of the Skin

Most observers have failed to find skin lesions in the rat comparable to those seen in man. Wolbach and Howe noted atrophic hair follicles and sebaceous glands but not keratinization of the follicular epithelium. Older rats (more than four months old) quite regularly develop scabby ears and tails, sores of the snout and feet and ragged fur. Recently Sullivan and Evans and Molt have reported hyperkeratotic lesions similar to the human ones. These were believed due to improvements in the basal diets used *in their experiments.*

Organs of Reproduction

Vaginal lesions lead to abnormal numbers of cornified epithelial cells in vaginal smears.

Evans and Bishop recommend examinations of vaginal smears as a dependable and early criterion of vitamin A deficiency. Success and failure with this method have been reported by others. The recent studies of Mason seem to explain the failures and to establish the test on substantial ground. *Mason observed that the first effect of the deficient diet was to*

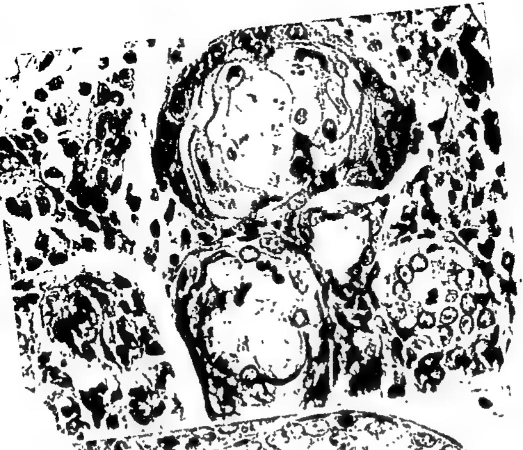


Fig. 2

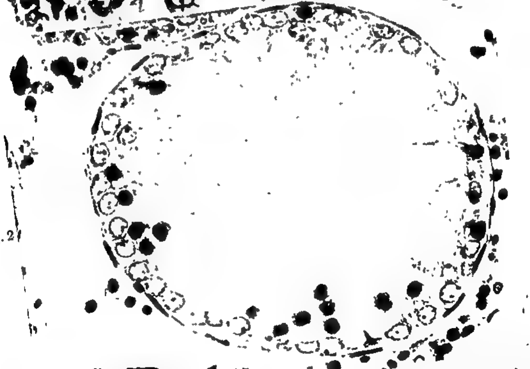


Plate IV Fig 1 Metaplasia in a small pancreatic duct as a consequence of vita-min A deficiency Lesions of this kind are very common in the pancreas in experi-mental vitamin A deficiency Fig 2 A seminiferous tubule showing the effects o vitamin A deficiency in the rat Spermatogonia and spermatocytes alone remain The latter are limited to two small clusters of cells. The lesion is typical of th late effects of vitamin A deficiency

exaggerate and prolong the appearance of cornified cells during estrous. Later the cornified estral phase becomes more and more prolonged until the entire cycle is characterized by cornified vaginal cells. In other words, the estral cycle must be followed in the early stages of deficiency to reveal the first pathological changes.

Pregnancy exaggerates the inherent weakness of the genital organs when vitamin A is deficient. Intrauterine death and resorption occur when the deficiency is exaggerated.

The site of the lesions is unpredictable with here and there along the horns of the uterus a diseased implantation site. The histological changes are, however, very constant

The maternal decidua becomes necrotic and infected, and the consequent interference with foetal nutrition leads to death. The damage is primarily to maternal cells in distinction to deficiency of vitamin E in which the first effect is on the foetal tissues. Vaginal hemorrhages with necrotic cellular debris, often with foul odor, announces the occurrence of fetal resorption.

By increasing the vitamin A intake somewhat, just sufficiently to keep animals on the border line of the earliest lesions of vitamin A deficiency, a variety of other abnormalities may be produced including prolonged gestation, difficult labor, sometimes with death of foetus or mother, retained and diseased placentae with areas of hemorrhage and leucocytic infiltration and a high mortality among the new born during the first five days of life. Difficult labor seems in part to be due to the cornified vagina which makes the expulsion of the foetus difficult, and to loss of tone of the abdominal muscles. Whether the latter is due to degeneration of skeletal muscle as described by Wolbach, with loss of striation, swelling and degeneration of fibers, is not determined.

It cannot be considered established that the effect of the deficiency operates directly on the decidual cells since degeneration of them has so far been seen only in association with infection. The degeneration may, therefore, be the result of infection.

Persistence of the epithelial cells often occurs between trophoblast and decidua and associated with necrosis and inflammation. Mason, to whom we owe these studies and description, considers that the deficiency has caused these epithelial cells to undergo degenerative changes which have prevented their digestion by the trophoblast.

The high mortality rate among the new-born may be due to poor mammary function as well as weakness of the young. Mammary function is inconstantly present and may be due to morphological changes in the breast since these are characteristically inconstant. Presumably congenital defects, as well as weakness, may appear in the young. Hale has extensively studied this phenomenon in swine and found microphthalmia,

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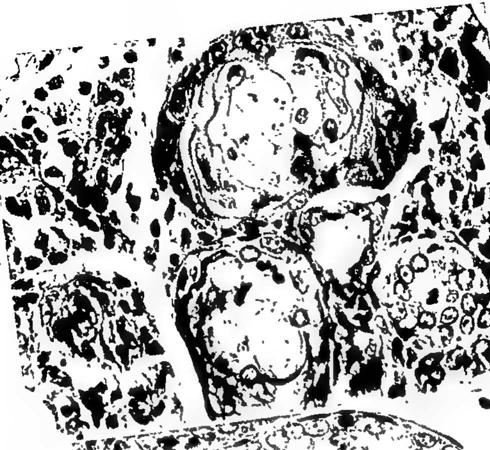
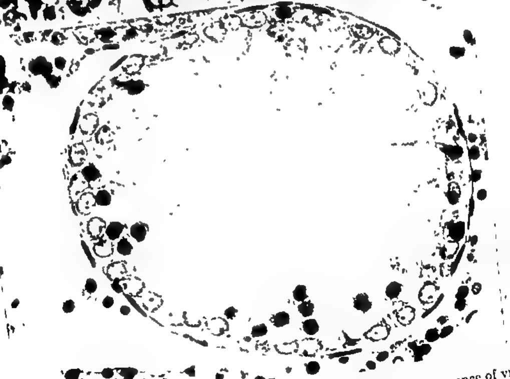


Fig. 2



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with the associated glands may well produce disturbed function. Richards believed it was primarily affected and found pits and erosions to be common throughout the bowel as well as hemorrhages and duodenitis and colitis. This has not been the experience of others. Hyperkeratosis and ulceration of the forestomach of rats has been observed by various writers since first described by Pappenheimer and Larimore. They believed that ingested hair had much to do with the development of the lesion. More recently Andervont has shown that strain differences are important in the etiology of this lesion. The reports of Howes and Vivier and of Sharpless indicate that the lesion is due primarily to a deficiency other than vitamin A. Thus Howes and Vivier demonstrated that yeast supplements were preventive and Sharpless that a supplement of flavine, nicotinic acid, cystine and rice polishing concentrate was likewise preventive.

One consequence of the study of gastric hyperkeratosis has been a number of experiments designed to determine whether this lesion plays a part in the causation of gastric carcinoma. Positive results were claimed by Fujimaki but others have failed to duplicate his results. A comprehensive investigation has recently been described by Fridericia and associates from Fibiger's laboratory. Fridericia and his colleagues found papillomata more common in deficient rats but were unable to produce carcinoma. Even the papillomata appeared not wholly or directly due to vitamin A deficiency since they occurred in some of the control animals. It may be that the immediate mechanism in keratosis of the stomach is vitamin A deficiency, that other factors induce a local deficiency effect such as we have discussed elsewhere. The character of the lesions suggests this.

Pituitary lesions, including an increased number of Alpha cells of the anterior lobe, have frequently been noted in cattle. Madsen describes these lesions in some detail and illustrates the serous cysts which form between the anterior and posterior lobes in young animals depleted of vitamin A. Many became blind and papilledema was sometimes observed. Madsen considers the lesions irreversible. Creech and Seibold reported on spontaneous vitamin A deficiency in cattle and described serofibrinous infiltrations of the subcutaneous tissue and muscles and intimal thickening of the peripheral blood vessels.

Visualization of Vitamin A in Tissues

An extremely promising technique in studying the lesions caused by deficiency is afforded by fluorescent microscopy. Several articles by Popper will be found valuable. Popper demonstrated that the fluorescing droplets contain vitamin A and studied their distribution among cells and organs. The Kupffer cells of the liver contain vitamin A and the amount is increased during febrile disease. Inflammatory lesions altered the dis-

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tribution of the vitamin. In the hepatic epithelium the amount varied but was reduced by exhausting disease, acute hepatitis and cirrhosis. Droplets are common in the adrenal cortex, in the corpus luteum and the Leydig cells of the testis. They appear in the kidney epithelium only in association with nephritis (Plate V).

Goerner and Goerner have described instructive studies which also penetrate the cell in exploring the rôle of vitamin A. They were able to show that dibenzanthracene, a carcinogenic compound, caused decrease in the amount of hepatic vitamin A. The vitamin A of the mitochondria of the liver cells disappeared and large amounts of vitamin A administered parenterally failed to replace this supply. Tumor cells likewise lack mitochondrial vitamin A. These studies have the further significance of exposing a disturbance of vitamin A metabolism in individual cells rather than in organs or the animal as a whole. It should be pointed out however that the liver store of vitamin A is relatively independent of hepatic function. Thus while many functions of the liver are affected by butter yellow rats receiving toxic amounts show no decrease in hepatic vitamin A (Clayton and Baumann). The same is true of individuals who have gastro-intestinal cancers and whose liver function and plasma vitamin A concentration are both abnormal. In such individuals the liver storage of vitamin A is not affected (see Vitamins and Neoplasia).

"HYPERVITAMINOSIS A"

A variety of lesions have been produced in young rats by feeding very large amounts of certain vitamin A concentrates. These are described as constituting "hypervitaminosis A." Among the lesions which have been most frequently described are trophic changes in the skin, bones and bone marrow. The skin lesions appear first. The hair becomes coarse and dull and later, commencing at the nose and ear margins, falls out. Ring crusts form on the tail. Thick secretion and inflammatory changes appear in the conjunctiva and rhinitis is often associated. The skeletal lesions include atrophy of the long bones which become very thin and brittle. Spontaneous fractures are common. These lesions were first reported by Collazo and his associates and have since been found by a number of other investigators. Rather different lesions were described by Drigalski; nephrosis (which was the usual cause of death) and toxic degeneration of the striated muscles and of the testis. Drigalski also observed the nasal and eye lesions. General agreement exists of the effect on the blood. A hypochromic anemia occurs with signs suggesting retarded erythrocytic maturation and a granulocytopenia. In most of these experiments a German preparation ("Vogan") was used. The report of Chalier and Jeune shows that the French concentrate "Amunine" is capable of essentially the same

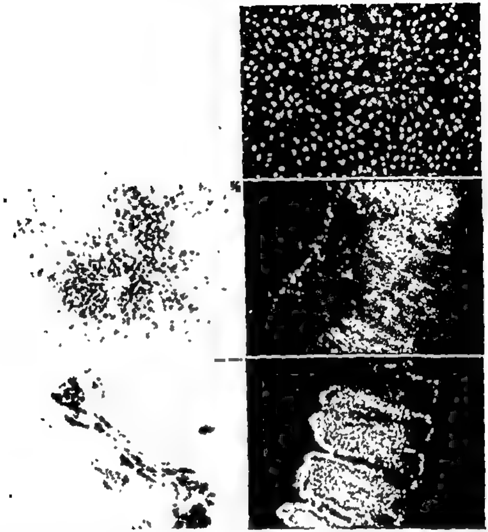

PLATE V. Distribution of vitamin A in various organs as shown by fluorescent microscopy. A. Liver of vitamin deficient rat. In the center is a shadow of a vessel. B. Liver of rat after feeding of large doses of vitamin A. The vitamin A fluorescence is imparted chiefly by the enlarged Kupffer cells. C. Normal human liver. Vitamin A fluorescence is imparted by the Kupffer cells and by fine fat droplets on the edge of the liver cells. D. Adrenal of normal rat. Vitamin A fluorescence is imparted by fat droplets in the epithelial cells of the fascicular layer of the adrenal cortex. E. Ovary of normal rat. The interstitial cell cords are rich in vitamin A. The corpora lutea show slight amounts. F. Intestine of rat after feeding of large doses of vitamin A. Vitamin A fluorescence is imparted by the epithelial cells and the lamina propria of the villi and by the lymphatics. (Courtesy of Dr. H. Popper.)

effects and Vedder and Rosenberg verified Collazo's observations by using jewfish oil, a rich natural source of vitamin A.

These observations are important in showing that the toxic agent which most investigators have felt to be something other than vitamin A, is a rather common property of fish oil concentrates. The opinion that vitamin A itself is responsible is based on two observations; the toxicity parallels the vitamin A potency and the destruction of vitamin A by irradiation also destroys the toxicity. Vedder and Rosenberg refute the first claim by showing that different jewfish oil preparations vary widely in toxic effect and that this is not related to the vitamin A potency. They believed to have demonstrated that molecular distillation eliminated the toxic substance or substances. Vitamin D and more strikingly 5 mgs. ascorbic acid effectively counteracted the harmful effects of jewfish oil.

Collazo's results are confirmed by Wolbach and Bessey who describe the skin lesions as similar to those seen in pyridoxine deficiency. The changes in the skeleton include drastic decalcification of the long bones and vertebral column, and increased numbers of osteoclasts. In the early stages osteoblastic and fusiform cells in the periosteum were found in increased numbers as well as small subperiosteal hemorrhages and accelerated cartilage growth at sites of endochondral bone formation. While the osteoblasts were numerous, osteoid tissue was deficient. No lesions were found in the teeth or internal organs but only a preliminary announcement has been made of the results of the investigation.

Cornil, Chevallier and Paillas found focal liver necrosis, enlargement of the islands of Langerhans, congestion and pigmentation of the spleen, desquamating keratosis of the skin and hyperactive spermatogenesis in guinea pigs given excessive amounts of vitamin A. Light, Ascher and Frey find hypoprothrombinemia, controllable by vitamin K, an effect of vitamin A overdosage (Science, 100: 225, 1944).

Thyroid Gland and Vitamin A

An association between the thyroid gland and vitamin A was suggested by early experimenters. Rabinowitch reported that the action of iodine in hyperthyroidism could be increased by adding a small amount of vitamin A. Fraser and Cameron confirmed this the same year. Extensive studies were reported the following year by Abelin who supplied various confirmatory evidence such as the increased vitamin requirement in animals given thyroxin. Later Wendt declared these two agents are antagonistic and reported that hyperthyroidism could be successfully treated by cod liver oil alone. Cases of exophthalmic goitre were shown to have abnormally small amounts of vitamin in their blood plasma and these returned to normal after thyroidectomy. Wendt's handling of these cases included the use of 50,000 to 80,000 units of vitamin A t.i.d. and blood determina-

tions to insure a high level (5 to 10 Lovibond blue units per 10 ml. blood). *Schulze and Hundhausen* added experimental studies which confirmed the general belief that thyroxin increased the vitamin A requirements.

There are some reasons to believe that the formation of vitamin A is dependent on thyroid function. Thus removal of the thyroid gland of goats causes the milk to become pigmented (carotene).

We have noted metaplastic changes in the thyroid glands of deficient rats.

MORBID EFFECTS IN MAN

The histological changes which follow deprivation of vitamin A in humans are indistinguishable from those experimentally produced in other mammals. However, species differences modify the location of the lesions, and the precise sequence in which they appear in man has not been established because the descriptions have so far been altogether too sketchy and few in number. Possibly differences exist in this respect.

Including the case reported by Leber in 1883, thirty post mortem examinations have been recorded as well as various isolated lesions. In all but four of the autopsied cases, the subjects were infants. Following Leber's report came that of Wilson and Dubois in which a well advanced stage of deficiency must have existed since widespread lesions were found. In addition to the keratomalacia, which had been repeatedly described, typical epithelial lesions were found in the bronchi, pancreatic ducts, uterus, trachea, and submaxillary glands. The bronchial lesions had resulted in numerous bronchiectatic cavities and the pancreatic lesions in retention cysts.

The third recorded case was similar, and was reported by Thatcher and Sure. The patient was a breast fed infant (also true of one case studied by Sweet and K'ang).

Subsequently, two series of cases have been published. *Blackfan and Wolbach* described the results of eleven post mortem examinations, including seven cases in which the diagnosis had not been made before death, but was established only when epithelial metaplasia was demonstrated histologically. Particular significance should be attached to this feature of their experience since it seems to promise that the diagnosis has frequently been overlooked, and that morbid effects of vitamin A poor diet are not uncommon in this country. The second series of cases has been described by Sweet and K'ang from Chinese subjects. In these the diagnosis had regularly been made before death from the presence of ocular lesions. One case had recovered from the effects of the deficiency before death from other cause, and another case was questionably one of deficiency since diphtheritic conjunctivitis was also present and masked the eye changes and no epithelial lesions were found at the autopsy. In six of

Fig 1

Fig 2

PLATE VI Fig 1 Extreme keratosis of the mouth of a sweat duct (right) and hair follicle (left) from a case of vitamin A deficiency Fig 2 Hyperplasia of the epidermis in a similar case (Both photographs furnished us by Prof C N Frazier)

–including the treated one—no metaplastic changes were found search seems to have been thorough.

es of victims of vitamin A deficiency are usually wasted. Abun- tores are rarely associated with the disorder (Blackfan and Retarded growth is very common, and atrophy of the lym- ne and degenerative lesions of the skeletal muscles are found. consist of swelling, loss of striation, and complete degeneration, calcification.

e effect of lack of this particular food factor probably expresses despread changes in the body, the specific lesions are found in njunctival membranes, the epithelium lining various organs and he skin and its appendages.

Ocular Lesions in Man

ar lesions commence as small, dry, round or triangular patches thi—Bitôt's spots. They are formed of cornified epithelium bacteria, very commonly *c. xerosis.* Cornification of the romptly followed by changes in the middle layers in which the e swollen and poorly stained and later vascularized. Infection, and corneal destruction may then occur, sometimes extremely

hanges occur in the conjunctival epithelium. The appearance l cells is probably preceded by the involution of the mucus ements. The conjunctival lesions are usually associated with and hyperemia.

and prominent expression of the deficiency in the eyes, at least a peculiar pigmentation which Pillat has studied in great detail, us modern techniques for the demonstration of precursors of The pigment is preponderantly of epithelial origin and probably m the protoplasm of the corneal epithelium. It is a defensive the damage done by light to cells damaged by vitamin A defi- llat has repeatedly emphasized the general pigmentation in his this does not seem to have occurred except in China. He con- kin pigmentation analogous to the corneal pigmentation though glands may contribute. In a number of his cases, the patients cases of Addison's disease. The pigment is melanin.

Skin Lesions in Man

lesions of animals have already been described. In man, dry- ess, furunculosis, scalp abscesses and bleaching or loss of hair, sent. The hair is also very dry. The follicular lesions, which elpful in establishing a diagnosis, vary in size to a maximum

diameter of 5 mm. They are hard, deeply pigmented and surrounded by a zone of pigmentation.

The center of the lesion, the papule, is a scaly, pointed plug of keratinized epithelium which can be expressed leaving a huge crater. While comedones are common on the face and are distributed like those in acne, the keratinized lesions do not occur on the face, and the two are never associated, though both respond promptly to treatment with vitamin A. This suggests a lesser functional disturbance of the sebaceous glands of the face, and a more advanced organic lesion in the glands elsewhere.

Histological examination shows hyperplasia and hyperkeratinization of the related epithelium of the hair follicles with metaplastic changes of the sweat ducts and degeneration of the glands—accounting for the dryness of the skin. Occasionally the base of the hair follicle becomes cystic from retained epithelium and separates from the remainder of the shaft.

Internal Organs

The most frequently affected internal organs are the trachea and bronchi and the pelvis of the kidney. In Sweet and K'ang's records, the trachea was found to have areas of metaplasia in six instances, the bronchi twice, and the pelvis four times. Other organs occasionally affected were: esophagus, three times; larynx, twice, and the uterus, tongue, pancreas and prostate, once each. Blackfan and Wolbach found metaplastic changes in all their cases.

In one child the nares and accessory sinuses were affected. This was the only case in which these tissues were examined.

The histological structure of the lesions described by all observers of the disease in man are identical with those in experimental animals. There is no need, therefore, to recapitulate the description.

Hemosiderosis of the liver and spleen are commonly associated with vitamin A deficiency. Sweet and K'ang found these changes in 50 per cent of their cases. Blackfan and Wolbach also mention atrophy of the bone marrow.

No satisfactory explanation of the diarrhoea, which commonly occurs with *xerophthalmia*, has been given. Cramer describes profound atrophy of intestinal villi in experimental animals, but Sweet and K'ang found no analogous lesions in the human gastro-intestinal tract.

Pneumonia has always been a common associate of vitamin A deficiency. Of the seven cases in which Blackfan and Wolbach were able to recognize vitamin A deficiency only after autopsy, five had died of bronchopneumonia. Lobular pneumonia was present in eight of Sweet and K'ang's cases, and three more had pulmonary tuberculosis.

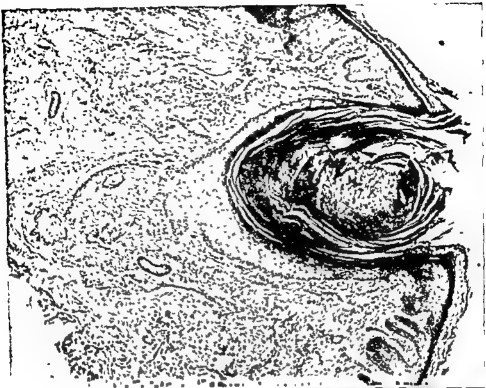

Fig. 1

Fig 2

PLATE VII Vitamin A deficiency in man A keratotic plug in a hair follicle The tip of the follicle is hyperplastic Frequently the tip of such follicles becomes cystic Fig 2 A well developed keratosis of the skin in a Chinese subject probably due to deficiency of vitamin A The photograph is of the elbow (Both photographs from the collection of Prof C N Frazier, Peiping Union Medical College.)

The relationship between estrogen and vitamin A seems more compl However we have found that if *sufficient* vitamin A be fed in conjunct with large doses of estrogen metaplasia does not occur, that the vitan appears capable of preventing what is otherwise a very uniform, prom and extreme lesion. Sherwood, Depp, Birge and Dotson report that la amounts of carotene and vitamin A *prevent the "normal"* cornification the uterus during estrus. They ascribed this to interference with t normal estrual cycle, to inhibition of the ovarian changes of estrus. T evidence for this interpretation is very inconclusive. It therefore see that in the case of uterine metaplasia, induced by estrogenic hormo as in trachea lesions incited by irritation, vitamin A plays the det mining rôle.

Is the stage of cornification in the normal uterus and the metaplasia estrogen overdosage to be considered a vitamin A deficiency lesion? If it is purely a local one for the other epithelial surfaces are not affecte This conception, of a local deficiency, has been independently suggest by Choun.

Choun reported 5 cases of giant-cell pneumonia in infants. This is rare disease which occurs in children between the ages of 6 months an 2 years. Clinically it resembles other forms of pneumonia but histological the lesions are quite peculiar, consisting of giant cells in the alveoli an thickened, hyalinized alveolar walls to which the giant cells are connecte These Choun interpreted as metaplastic alveolar epithelium. Seifried experience, to which reference has been made affords substantial eviden that giant cell formation may occur in the respiratory tract in associatio with vitamin A deficiency. In every case Choun was able to demonstrat metaplastic and hyperplastic changes in the bronchiolar epithelium as wel Choun suggested that the inflammation might serve to produce "a loca deficit in the vitamin."

It is true that in Blackfan and Wolbach's 13 cases of A deficiency i infants 7 were believed to have had an adequate diet and in 5 of thes the suggestion was made that they might be due to a disturbed metabolism from chronic generalized infection. Such a mechanism could operate through hepatic damage or other effects, to induce a systemic deficiency. But giant cell formation did not occur in any of these 13 cases. Choun's suggestion is an interesting one but the facts are still much too incomplete to justify any conclusions.

As far as estrogenic hormone is concerned there is other evidence that it is closely related. Zuckerman and Parkes produced metaplastic changes in the prostatic utricle of monkeys by estrogenic substance and recently Silberberg and Silberberg report that guinea pigs given large daily doses of estrogens develop skeletal lesions. The cartilage changes are described

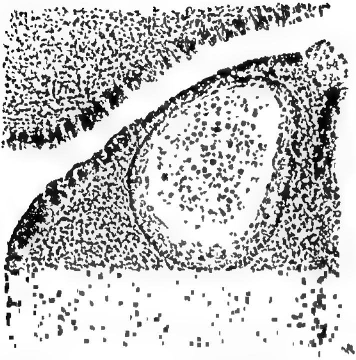

Plate IX. Effect of vitamin A on metaplasia induced by theelin. Photograph of the endometrium of a rat treated with theelin and maintained on a vitamin A deficient diet. Fourteen days before the animal was sacrificed it was given large doses of cod liver oil. The dilated gland has exfoliated keratinized epithelial cells. The basal layer of epithelium is extremely hyperplastic. This is the typical appearance of repair in vitamin A deficiency metaplasia and occurred despite the continued administration of theelin. (From McCullough and Dalldorf, Arch. Path., 24: 486, 1937.)

as "premature aging," the growing end of the bone is replaced by a bony plate. These changes, as far as can be judged from the illustrations and description, are very much like those of vitamin A deficiency. *No mention* was made of the condition of the epithelial tissues or the diet used.

THE DIAGNOSIS AND TREATMENT OF AVITAMINOSIS A

The large outbreaks of avitaminosis A have been associated with extreme privation, as among the poor of China, with wars and sometimes with special circumstances such as existed in Denmark during the First World War when the price of butter and milk rose to such outlandish levels due to the insistent demand by Germany for butter fats that the Danes stopped using milk products.

Age Group Reactions

The disease occurs in all age groups and has been seen in the new born as well as in persons of advanced age. The majority of cases occur in infants, however. The precise period of infancy varies with the dietary habits of the people. In the Danish epidemic the infants were bottle fed on skim milk and the average age was less than in the Japanese cases in which the deficient diet was given after weaning. Among the 203 cases reported by Sweet and K'ang a second peak of incidence occurs in early adult life. This is ascribed to the impoverished diet prevalent among soldiers and apprentices in China. Cases secondary to gastro-intestinal or biliary lesions may, of course, occur at any age although the requirements of infancy are apt to be more imperative and relatively greater.

The epidemics of vitamin A deficiency in Europe during the World War occurred mainly in the winter months, Birch-Hirschfeld's cases appearing between November and February The peak incidence in China occurs during the same season although a smaller rise was found in summer which was attributed to dysentery and consequent depletion.

Judged by the information we now have the epidemics of vitamin A deficiency have been predominantly marked by eye or skin lesions In some instances the separation has been so sharp that one might doubt whether the two are related. Yet in many instances this has been only a matter of emphasis and in certain cases both manifestations have been present. Most of Frazier and Hu's patients who had keratomalacia also had skin lesions although the reverse was not true. Of Loewenthal's 81 cases of deficiency 74 had dermatosis, 45 had xerophthalmia and 71 hemeralopia. A review of many reports indicates that there is a considerable degree of correlation between age and the predominant lesion. Eye lesions have been more common in children and severe lesions leading to corneal ulceration in children under 5 years of age.

The medical history usually reveals diets which have been extremely poor, or completely lacking, in animal fats and green vegetables, either of the patient or, if a breast fed infant, of the mother.

The incubation period is variable, depending on the degree of deficiency and the previous intake of the vitamin. It also varies with the age of the patient. Forest and Wolff estimated the depletion period in very young infants as three to four months, while twice as long a period has occurred in children more than twelve months old.

Infant cases are usually stunted, with a dry scurfy skin less sensitive than normal, sometimes with loss of hair and frequently with skin infections. Diarrhoea is common and many cases have come under observation because of complications, usually bronchopneumonia. In Ceylon, the affliction is called "mandama" and the four characteristics are said to be xerophthalmia, stunted growth, diarrhoea, and a toad-like skin which Nicholls calls phrynoderma.

Bloch said it was rare for a child to appear well and develop xerophthalmia though occasionally an infectious disease seemed to precipitate it. The children were first indolent and then irritable. Anemia and latent or manifest edema are common.

Night-blindness and Xerophthalmia

The first definite symptom is night blindness—hemeralopia. This is seldom recognized in very young children and it sometimes develops so insidiously that it is recognized late in adults. Patients complain that they are unable to get about in darkened, but familiar surroundings. In a recently recorded case, the patient had complained to the civil authorities that the street lights were defective. Exposure to strong light aggravates the defectiveness of vision in poor light, and vision is better in the morning than in the evening because of exposure to light during the day.

An extensive study of mild cases of hemeralopia has recently been made by Fransden and is of interest because of his analysis of the symptoms present in such cases. Common complaints were difficulty in sewing and reading at night unless the light was brilliant, dancing of letters on the page, poor vision exaggerated by weak light, photophobia, glittering images, and dancing specks—muscae volantes. Less frequent symptoms were altered sensitivity—paraesthesias—, muscular twitchings of the eyelids and extremities, nervousness, dryness of skin and mouth, pelvic pains, and decreased sweating. We may assume from this, that some of his patients had other symptoms of A deficiency than the hemeralopia alone.

The signs of the disease in the eyes are a patchy dryness and Bitôt's spots. These latter resemble dried foam, are gray or light yellow in color, impervious to tears, and look as though they might easily be brushed off.

Loss of luster and wrinkling of the conjunctiva follow. Conjunctivitis and photophobia are often associated with the early lesions, and in some cases mask the more important changes. A light brown pigmentation throughout the conjunctiva is also characteristic as is reduced sensitivity of the conjunctiva and cornea, which may persist for months after all other signs have disappeared. Usually both eyes are involved and at about the same time. In older children a viscid, stringy conjunctival secretion is present and the lids are stuck together. When this is cleaned away the cornea may still be clear but photophobia and blepharospasm are complained of. As the disease advances the Bitôt's plaques appear and may cause adhesions between lid and eyeball (Yudkin). One or more small ulcers may occur on the cornea and these progress to perforation. However, older children usually have only a xerosis conjunctivae. Hypopyon is characteristic of the disease in infants at which time of life the lesions develop rapidly.

These manifestations are all dependent on the fundamental alteration in the surface cells which precedes the macroscopic changes. The Bitôt's spots are areas of thickened epithelium; the conjunctivitis is dependent upon degeneration of the mucus secreting cells, the roughening of its surface which harbors bacteria, and probably impaired tear glands and ducts which reduce the cleansing action of tears.

If the condition is allowed to progress the cornea becomes gray and opaque and softens. These lesions may be solitary, but in other cases many small foci liquefy simultaneously. An effusion of pus—hypopyon, prolapse of the iris, and even general inflammation of the eyeball—panophthalmitis—are late effects.

The progress of the eye lesions is less rapid in adults in conformity with the essential differences in other aspects of the disease in the young and in the full grown. Blindness is a common result of vitamin A deficiency and, particularly in the young, treatment must be begun early in the disease before corneal opacity occurs.

Skin Lesions

Current interest in the skin manifestations of vitamin A deficiency may be credited to the investigations of Nicholls in India, Loewenthal in Africa and Frazier in China. Nicholls and Frazier both deduced that the dermatosis was a very early sign of deficiency, an observation in good accord with later evidence. Nicholls found advanced cases without evidence of ocular disease and Frazier and Hu reported cases in which the skin lesion was seen to precede eye signs by several weeks. The most complete description has been supplied by Frazier and Hu.

The earliest change is an abnormal dryness of the skin due to suppression

of the sweat glands. This is followed by keratosis which is most extensive in the hair follicles which become filled with dry, hard, pointed papular masses of keratinized epithelium and may be very irritable. These lesions are commonest on the antero-lateral aspects of the thighs, the postero-lateral aspects of the forearm and gradually extend to the extensor surfaces of the shoulders, the lower abdomen and sometimes to the chest, back and buttocks. The papular masses are of a dark, dirty color and are frequently surrounded by a zone of greyish pigmentation. The skin between is dry and roughened. Various observers have remarked that the condition can be more easily recognized by palpation than by inspection. The scaliness gives a whitish coloration to the skin of negroes who have said they "ash" (Lehman and Rapaport).

Frazier and Hu found age to be a determining factor in the appearance of these skin lesions. Of the cases of vitamin A deficiency observed in the Peiping Union Medical College only 2 per cent of the cases of keratomalacia under 15 years of age had dermatosis while 30 per cent of the adult cases had characteristic lesions. They felt that before puberty vitamin A deficiency results in a condition of simple xerosis, after puberty in xerosis and spinous follicular lesions. This would explain the absence of similar lesions in the Danish epidemic which was limited to infants. However Aykroyd and Rajagopal found 129 typical cases among Indian children of school age and one fourth of the 4,380 children examined by Nicholls had similar lesions. Xerosis is more common but follicular lesions also occur in children. It seems reasonable, although by no means certain that those instances of keratomalacia without follicular lesions represent a more severe and acute deficiency, exaggerated by the more insistent demands of rapidly growing infants and that the follicular lesions require a considerable time to evolve just as they are very slow to heal.

In certain cases lesions have also been seen on the face. These superficially resemble acne. Youmans and Corlette, in describing 11 patients they observed in Nashville, remark that in addition they found other patients with lesions having the same distribution and essentially the same microscopic structure but associated with an inflammatory reaction. Many of the individual lesions looked pustular or acneform although no frank exudate could be expressed from them. The lesions were not as elevated as the spinous ones described by Frazier and Hu and the skin was not dry. Nevertheless these atypical lesions responded to treatment with vitamin A. The evidence of night blindness and of xerosis conjunctivae was indefinite in Youman and Corlette's cases. This was interpreted to mean that the skin lesions preceded the eye signs. The significance of the acneform lesions is still uncertain. They were not uncommon in Loewenthal's cases for he wrote that the skin "presented the clinical picture of

acne vulgaris combined with a dermatosis which none of the medical officers present could define." Loewenthal's solution of this problem is all the more creditable because his cases were complicated by other deficiencies. Neuritis, itchy scrotum, mouth lesions, diarrhea, general infections, cutaneous sepsis and changes in the hair. These he distinguished from the cardinal signs of vitamin A deficiency, xerophthalmia, keratomalacia and night blindness. "At subsequent monthly inspections of prisons, all new cases of this dermatosis were recorded, and it was found that the majority of these men suffered from night blindness and xerophthalmia, while almost every sufferer from xerophthalmia and night blindness showed these cutaneous changes."

Thus evidence concerning the striking character of the skin changes associated or not associated with xerophthalmia was accumulating and older reports took on fresh meaning Nicolau's report of keratotic lesions with associated acneform ones having the distribution and minute structure of Frazier and Hu's, Loewenthal's and others cases may now be interpreted as due to vitamin A deficiency. Nicolau ascribed these cases to scurvy, they occurred in Roumania during a scurvy epidemic (1918) and were sometimes associated with petechiae. Aschoff and Koch, in their classic study of the same epidemic, dismissed the idea that they were scorbutic lesions. "These elevations have nothing to do with scurvy." The lesions were well known, according to Aschoff and Koch, to the Turks who called them "keratosis pilaris" and who reported that they were very common in Turkey. Aschoff and Koch designated these lesions as keratosis suprafollicularis and described them in sufficient detail to establish their identity with those of Frazier and Hu. They were frequently seen in patients without a single sign of scurvy. In this regard it is interesting to note, nevertheless, that according to earlier writers quoted by Aschoff and Koch, the lesions had long been considered an early manifestation of scurvy.

Keil has recently reported 2 cases of dermatosis associated with scurvy. One patient had ulcerative colitis, positive capillary fragility test and responded clinically to vitamin C. Her skin was generally very dry and hyperkeratotic areas occurred on the arms, thighs and abdomen. The hairs in the diseased follicles were fine, deformed and curly. Each lesion was surrounded by an area of hyperemia or of hemorrhage. The second patient had been on a grossly deficient diet. The lesions were identical. Keil refers to the older references to "lichen scorbuticus" or "scorbutic goose-flesh" and suggests it is a genuine manifestation of scurvy. It is not as marked as the keratotic dermatosis of vitamin A deficiency. Neither patient gave symptoms of night blindness.

More pertinent are the instances of typical dermatosis reported from our own country. Jeghers described lesions of the same type although of less

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sponse to large doses of vitamin A (200,000 Int. Units given orally). In 2 a marked improvement was detected within 2 hours. The second noteworthy feature of the cases of Lehman and Rapaport is that the distribution of the cases within the families suggested a hereditary influence. In one instance 2 sisters both had dermatosis while the parents and 3 other siblings were apparently normal although the mother had suggestive evidence of night blindness. In a second family the mother and 2 daughters had had the skin lesion for many years while the father had a normal skin (Fig. 34).

Follicular keratotic lesions may be classified histologically in several varieties. Kyrle, for example, separates ichthyosis follicularis from keratosis follicularis on the basis of hypertrophy of the stratum granulosum throughout the follicle wall and the absence of sebaceous glands. Broken or lanugo hairs are not present. Keratosis follicularis on the other hand involves only the mouth of the follicle and thus causes cystic dilatation of the distal portion of the follicle. Hairs are commonly present. The granulosa layer is thinned from pressure. It is this lesion which is most closely reproduced by vitamin A deficiency. Ichthyosis follicularis is a congenital condition.

Darier's disease, also called keratosis follicularis (or Psorospermosis follicularis), represents a more extreme degree of keratosis in which dyskeratosis as well as hyperkeratosis is seen. The lesions can become very large and are quite distinct from those of avitaminosis A although some cases improve under vitamin A treatment. Carleton and Steven report four cases among two families. One member of each family showed complete resolution following therapy. Ten patients were studied by Peck, Glick, Sobotka and Chargin. In eight the plasma vitamin A was low and the five tested for dark adaptation showed abnormally slow regeneration. Success followed treatment with 200,000 U.S.P. units. Remissions occurred when treatment ceased.

Skeletal Lesions in Man

It is surprising that so few applications of the known facts of vitamin A deficiency have been made in orthopedia conditions. A successful exception appears to be Scheuermann's disease or *kyphosis dorsalis juvenilis.* Following Schneider's suggestion that epiphyseal lesions are frequently related to vitamin A deficiency Simon treated a number of cases of Scheuermann's disease with plaster-of-Paris corsets and 40,000 Int. Units of vitamin A daily for three months. This regimen has also been used with excellent results by Cornell

Scheuermann considered the primary disturbance to be in the epiphysis but Schmorl interpreted the lesion as prolapse of the nucleus pulposus into

severity among medical students and Steffens, Bair and Sheard produce the lesion in a healthy individual by vitamin A depletion. More recent Lehman and Rapaport have identified 9 cases among children in New Yo City. Their report is of particular interest in two respects. In the fi

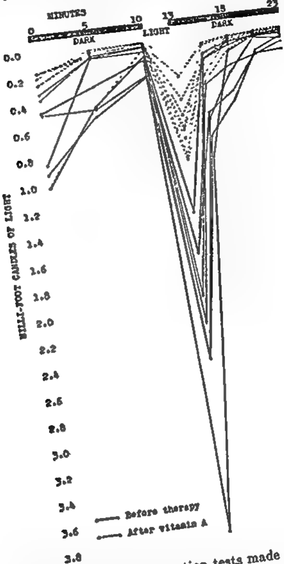

FIG. 34. The results of visual adaptation tests made with the "Biophotometer" on eight children before and after vitamin A therapy. Other signs of vitamin A deficiency were present in these cases. (Reproduced by permission of Dr. Edw Lehman and the J A. M. A. See Lehman, E., and Rapaport, H. G., J. A. M. A 114: 386, 1940.)

place they followed all of their cases with photometric tests for visual ada tation using the Biophotometer. In each patient subnormal readings we secured although ocular lesions were not recognized. In 4 of their patie these observations were fortified by determinations of the immediate

It seems likely that cases may be found in which the conversion of carotene into vitamin A is interfered with by liver disease, but use of carotene has been successful in most cases. Such limitations will be few. Faulty absorption of the vitamin and of its precursor, the factors making for further inhibition or lack of utilization, need much further study.

Wald, Jeghers and Arminio, measuring the threshold of the completely dark adapted eye, were able to correlate almost immediate response (7 minutes) to vitamin A given parenterally. Fisher reports a severe case of night-blindness in a boy. A single dose of 36,000 Int. Units of vitamin was given and the night-blindness responded within one hour. The conjunctival sacs became moist and distinctly improved within a week. Treatment was supplemented with cod liver oil instilled into the conjunctivae. Similarly Aykroyd and Wright reported children with keratomalacia showed arrest of the lesion in 1 week and definite improvement within 2 weeks. Vaillant and Gillis reported that a young girl almost completely blind at night given 10,000 Int. Units daily improved in several weeks.

The skin lesions respond more slowly. Youmans and Corlette seldom saw response within 1 month. Often 3 months was required. However Frazier and Li, who treated a young male adult with 1 to 2 mgm. of carotene daily (given intramuscularly) observed shrinkage in the papules within 1 week. By the 9th day many plugs were falling out of the gaping follicle mouths and a corneal ulcer began to heal. Three days later it was completely healed. The man's skin was much smoother within the month although a few lesions could still be found on discharge, 51 days after treatment was commenced. Nicolau's cases, treated by an adequate diet alone recovered within 3 months although pigmented areas remained. This has been noted by subsequent authors. Lehman and Rapaport's patients showed a very prompt response to treatment, as measured by the photometer test but required 2 months for resolution of the dermatosis.

Kirwan, Sen and Bose urge that vitamin A be given parenterally in children less than five years old. Their experience in India indicated that such treatment was justified by the danger of keratomalacia.

Special Aids in the Diagnosis of Vitamin A Deficiency

The special tests which have been suggested have been chemical measurements of the vitamin of the blood, methods of establishing the presence of metaplasia and means of identifying and measuring night blindness.

Night blindness can usually be determined from the patient's history if well developed. Simple procedures such as the use of a watch dial with luminous numerals will frequently confirm the diagnosis. In addition various special instruments have been designed and used for measuring the degree of dys-adaptation. Most of these are expensive and their use re-

the spongiosa. Roentgenographic examination shows flattened discs, wedge-shaped vertebral bodies, destruction of the epiphyses and cartilaginous nodules. The result is a rounded back, sometimes with mild scoliosis but without fixation. Most children with rounded backs are relieved by gymnastics but in Simon's series 1 per cent became worse under such handling and were treated by rest and vitamin A, the rest being provided by the plaster corset.

Other Symptoms

Hoarseness and a dry cough often occur and are probably due to reduction in the bronchial mucus secretion. Diarrhea is also common. Paraesthesia and motor disturbances have been reported but are presumably due to other causes. Of Pillat's cases 40 per cent had gastric hypoacidity and Pillat judged this was related to both the severity and the progress of the deficiency.

The common complications are pneumonia, pyelitis and cystitis. Herbert found desquamated epithelial cells, and often pus cells as well, in the urine of all of his cases.

Prognosis

Prognosis naturally varies with the severity and duration of the deficiency. In infants the mortality rate has always been high during epidemics of xerophthalmia. During the Danish epidemic it was 21 per cent. The incidence of blindness is, of course, much higher and stigmata of the disease, which affect life expectancy, remain after recovery. In Bloch's patients, many of whom were kept in homes and institutions for blind children where their progress could be observed, it was found that one-third died before their 8th year. The milder cases without eye lesions recover promptly under proper treatment.

TREATMENT

The treatment consists in giving vitamin A in adequate amounts in the diet and by use of concentrated preparations. Cod and halibut liver oils, A concentrates and carotene, are all suitable. In cases where an obstructive jaundice co-exists, bile salts must be given with the vitamin for its absorption depends upon their presence.

Since human cases have almost invariably developed after diets deficient in several factors, a well planned, complete dietary routine is desirable. Vitamin A deficiency rarely occurs singly. Of the recorded cases in infants, the most common associated deficiency disease has been rickets, but perhaps that is only because symptoms of other vitamin deficiency were not recognized.

more certain if certain special examinations are made. According to Sweet and K'ang, the best procedure is to retract the eyelids for three to five minutes, watching all the while for the appearance of dullness or haziness, and then to wipe a spatula lightly across the conjunctiva and stain the cells secured. Keratinized epithelium and *c. xerosis* will be found early in vitamin A deficiency. This method is particularly suitable for infants, since it requires no cooperation from the patient.

Blackfan and Wolbach recommend the following criteria:

1. An inquiry into the food habits of the patient, particularly the supply of vitamin A containing foods. The tabulation of vitamin A potency of common foods in the Appendix will be found helpful in estimating such dietary adequacy.

2. Examination for morbid processes which might interfere with the use of fats, such as disease of the biliary system and pancreas, vomiting or diarrhoea.

3 Examination of the eyes for xerosis or night blindness.

4 Search for keratinized epithelial cells in scrapings from cornea, nose and mouth, the secretions of the vagina or in the urine They recommend staining by Gram's method with acid alcohol decolorization Sweet and K'ang felt the distinction between the buccal epithelium in health and deficiency was difficult to determine in scrapings.

Youmans and Corlette made and examined smears from the bulbar conjunctiva of normal and poorly fed individuals in an effort to establish criteria for the diagnosis of mild forms of deficiency. The cells were finally classified as nucleated or non-nucleated and the results showed no significant difference between the proportions of each type and the nutritional status of the patients. In none of the cases studied were definite signs of vitamin A deficiency present but several of the cases were believed to represent partial deficiencies. It seems apparent, therefore, that this criterion of deficiency is inadequate in partial states of depletion as we have been recently accustomed to think of them or will require an improved technique. That it is satisfactory in the diagnosis of cases with signs of the disease is well attested.

The biomicroscope has been extensively used, particularly by Kruse, in the detection of conjunctival changes unrecognisable by examination with the unaided eye. There is no agreement that changes characteristic of vitamin A deficiency can be identified in this way, although the skill and experience of Kruse may explain the more definite results be secured. To a pathologist it would seem much more reasonable to demonstrate early thickening and keratinization by cytologic means. Kruse's reports should be consulted as well as the results of Kodicek and Yudkin.

The determination of vitamin A concentration in the blood and urine has been used clinically. In these tests the usual procedure has been to use the antimony trichloride method described in the Appendix or a modification of it. The results naturally suffer from the well known weaknesses

quires special training. All operate on the principle that if the eye be first exposed to a brilliant light and the retinal pigments bleached the rate of recovery may be determined by establishing the threshold at which targets of variable brilliance may be identified. The first instrument of this type, the Birch-Hirschfeld photometer, was relatively simple, a diaphragm being used to regulate the intensity of the illumination of the target. The bleaching light was not provided. The values were purely arbitrary.

A popular successor to this instrument was the Biophotometer which incorporated the bleaching light within it and also attempted to express the intensity of the illumination in appropriate values. To much of the work with both these instruments the objections were raised that many patients learned to read the targets in dimmer light quite independent their actual adaptation and also that the changes reported after treatme could equally well be explained by chance (Fig. 34).

The phenomenon of dark adaptation was then thoroughly studied by Hecht and an improved instrument devised which tests a fixed retinal field (necessary because the proportions of cones and rods in different regions of the retina vary and therefore the response to dark adaptation tests) and incorporates superior control of the light values used. This machine has not been widely used but appears to afford the most precise means of determining and measuring dark adaptation.

However subsequent work has shown that the simpler instruments will also yield significant observations if their limitations are thoroughly controlled. Jeghers' results seem to be reliable and extensive work by Booher and Williams is encouraging. These studies were made with the Biophotometer. Even simpler instruments are satisfactory. Pett has described a simple device which he found suitable. The reader should consult the reports of Hecht and Mandelbaum, Jeghers and Pett.

A clear understanding of the phenomena involved is required. We know of no better description than that of Hecht.

"Night blindness is a vague term and has to be replaced by quantitative statements about dark adaptation and visual thresholds. The visual threshold at any moment is the light intensity required just to elicit a visual sensation, and dark adaptation is the change which the visual threshold undergoes during a stay in the dark after an exposure to light. The change is enormous; after exposure of the eye to daylight, a stay in the dark for thirty minutes results in a decrease in the threshold by a factor of a million. The change is made in two steps, the first being mediated by the retinal cones, and the second by the retinal rods. The first is rapid and is over in three to four minutes; the second is much slower and takes about twenty-five minutes."

The diagnosis of manifest cases of vitamin A deficiency can be made

CHAPTER XVII

THIAMINE DEFICIENCY

All of the fractions of the vitamin B complex are heat stabile except niacin, thiamine and biotin. Biotin deficiency has not been demonstrated in man excepting under highly unnatural conditions but thiamine deficiency is very common. The most serious form is the highly characteristic and thoroughly studied disease called beriberi which is most prevalent in the Far East. Our own nutritional habits afford sufficient thiamine to prevent beriberi but mild, atypical forms are very common and many nutritionists believe that most of us do not consume sufficient thiamine for optimum health.

Deficiency of thiamine in many mammals and birds results in disease having all of the cardinal signs of human beriberi. The lesions are also similar to those in man consisting of degenerative changes in the nerves and enlargement of the heart. The pathological physiology of both spontaneous and experimental thiamine deficiency is more specific than the lesions and of recent years more emphasis has been placed on the functional than the anatomical consequences of thiamine deficiency.

The amounts of thiamine required to prevent beriberi are well known. They are, as in other vitamin deficiencies, much less than is necessary to saturate the tissues. The considerable gap between these two values remains an uncertain field of knowledge and the minimal requirement for optimum health is not yet known. Dietary requirements are modified by the protein and fat value of the diet and presumably by synthesis in the intestinal tract.

Special clinical interest attaches to forms of thiamine deficiency due to disease and the relationship between various peripheral neuropathies and diet. In addition there are uncommon but severe diseases, in addition to beriberi, which respond well to thiamine administration.

BERIBERI

(*barbiers, hinchazon, maladie des jambes, kakke, suba, loempoe, taou, maladie des sucreries*)

Beriberi is important for the harmful effect it exerts on the health of a large part of the world. It is of particular historical interest because it was the challenge which prompted the modern [illegible]

[illegible]

in our own country.

of the test which does not measure the vitamin A value at different le of concentration with any considerable accuracy. However it is useful the degree that cases of severe depletion, as reported by Haas and Meu mans, demonstrate a complete absence of blood vitamin A. Serum car tene, which is simply and accurately measured seems to bear no clos relationship to the vitamin A value and carotene was present in all of the samples tested by Haas and Meulemans. Quantitative deductions from serum vitamin A do not seem to be justified.

The presence of vitamin A in the urine has not been correlated with vitamin A nutrition. The vitamin is present only during diseases which affect the liver and might be considered more a test of liver function than of vitamin A nutrition. Vitamin A spill does not occur in the urine even after massive doses unless the liver be damaged (Schneider and Weigand). Wendt has improvised a tolerance test in which the intestinal elimination of vitamin A is measured and used as a yardstick to determine when saturation has occurred.

beriberi appeared in 1882 when Bälz and Scheube reported clinical-pathological observations made in Tokyo and Kyoto. It is amusing to note that Bälz characterized beriberi as endemic polyneuritis of the kind seen in Europe in sporadic cases (Vedder). Since that time many competent workers have studied all phases of the disease. North American interest was aroused when beriberi became a problem in the Philippines and R. R. Williams, whose sustained efforts were eventually rewarded by the synthesis of thiamine, became interested in the problem there.

It was natural that the disease was first suspected to be an infection. It appeared frequently in epidemic proportions and the infectious nature of disease was new and promising. The absence of cases among Russian soldiers during the Russo-Japanese War, when the Japanese suffered greatly, and the immunity of hospital attendants cast doubt on an infectious etiology and attention turned to the explanation that a toxin was responsible. This opinion was shared by Eijkman, who first produced the experimental disease, and who believed that rice polishing was therapeutically active because it contained an antidote.

Rice had been implicated from the beginning and Takaki, faced with the problem of epidemic beriberi in the Japanese Navy, ordered the ration changed replacing much of the rice with other foods. This program reduced the incidence of beriberi from more than 23 per cent to less than 0.5 per cent. It is interesting to note that the measures Takaki took not only increased the thiamine content of the diet but, by providing more protein and fat, exerted a sparing effect on the thiamine.

The chain of events which led to the discovery of vitamin B_1 began when the Dutch Government sent a commission to the East Indies to investigate beriberi. The commission was in charge of Professors Pekelharing and Winkler who were assisted by Christaan Eijkman, a young army doctor. The commission was prepared to find beriberi an infectious disease (Pasteur's great discoveries were uppermost in mind) and did so. They isolated a micrococcus which they suspected to be the cause of the disease. When they returned to Holland Eijkman remained and spent the next two years attempting to prove the infectious nature of beriberi and the significance of the bacteriologic findings. Eijkman was working under stringent financial restrictions and the hospital where he had established his laboratory did not provide funds for the feeding of his animals. They were therefore fed on the kitchen scraps and to Eijkman's surprise many of his chickens developed paralysis and other symptoms suggestive of beriberi. The suggestion that the birds had been infected was dispelled by the observation that when their diets were altered they promptly recovered. Eijkman ascribed the recovery to an antidote in rice polishings, inferring that "polyneuritis gallinarum" or avian beriberi was caused by a toxin in polished rice.

Incidence

The incidence of beriberi is largely a matter of speculation, but is doubtless very great. Comprehensive health records are not available for those countries in which it is most common. It is endemic in China, India and Burma. In Hong Kong there formerly were 10,000 cases annually among a population of 350,000 persons. Fehily gave the incidence among children attending a welfare station (1941) as 18 per cent, and as recently as 1938 1,661 deaths in Hong Kong were ascribed to beriberi. Aykroyd and Krishman estimated that there were 40,000 cases each year in Madras.

A good deal more is known of the frequency of the disease in the Philippines and Japan. In the former country 18,000 deaths were due to beriberi in 1935. The official Japanese report in 1934 listed 11,841 deaths and Yanagi found 14.2 per cent of 888 clinic patients afflicted, approximately the same rate that had existed for ten years. One out of every ten hospital admissions was a case of beriberi.

Many of the deaths occur in infants. In the Philippines, where the infantile form is known as "taou" or "Suba" disease, it was for long the third most common cause of death in infancy. In Japan it is the eleventh commonest cause. These figures reflect both the incidence and malignancy of beriberi in infancy.

Fewer cases occur in Africa and South and Central America. In most of these areas it is sporadic, although it is said to be endemic in Brazil. Sporadic cases occur in Europe and isolated cases in the United States where 49 deaths were reported in 1941. All of these records tell little of the incidence of less severe thiamine deficiency.

The war has doubtless intensified the severity and increased the frequency of beriberi for millions of people. Reports are already reaching us of the prevalence of deficiency disease in concentration camps and famine areas and a concerted effort is under way to control these conditions when hostilities cease.

Etiology

The earliest records of beriberi occur in Chinese writings which include a clear description written in the 7th century. The disease first occurred in Japan a thousand years later when polished rice was introduced as a staple among the urban population. The first cases occurred in Tokyo (then Yedo) and were "Yedo-Wazurai." Osaka and Kyoto had cases somewhat later and it is said that patients were successfully treated with diet at that time.

Bontius described the disease in Batavia in 1627 and first used the term beriberi. The first description in the western hemisphere is that of Piso who observed cases in Brazil (1636–1642). The first thorough study of

are said to be normal. There is no tendency to bleed, no skeletal lesions. The failure of recent human experiments to elicit edema and cardiac dilatation may have been due to the brevity of the experiments. Excepting these a diet complete, by modern standards except for thiamine, induces symptoms identical with those of oriental beriberi as is shown in Table XXIX. In both thiamine promptly cures. In both the nervous symptoms are slow to respond to treatment and in both other dietary supplements do but

TABLE XXIX

Occurrence of Certain Symptoms in Experimental Thiamine Deficiency and in Spontaneous Beriberi

SYMPTOM	EXPERIMENTAL DISEASE	BERIBERI (ORIENTAL)
Fatigue ...	first symptom	present
Anorexia.	early	present during prodrome in 50 per cent
Constipation	present	present
Blood pressure	decreased	*diastolic decreased*
Tender calves	present	present
Bradycardia	present	characteristic of prison beriberi
Weakness of legs	present	present
Altered reflexes	present	present
Enlarged heart	not present	common
Edema	not present	common
Plasma protein	normal	normal
Serum calcium	normal	normal
Anemia	none	none
Tongue	normal	normal
EKG	altered	similarly altered
Sugar tolerance	decreased	decreased
Gastric acidity	decreased	decreased or increased
Gastro-intestinal motility	altered	altered
Effect of treatment	prompt	prompt
Adrenalin test	not done	positive
Work capacity	decreased	not tested
Blood lactic acid	increased	not tested

(Largely based on Williams, Wilder, Mason and Smith and Shimazono.)

little to hasten their resolution. It would seem that the slow response of the nerves is a characteristic of the lesion and does not have etiologic significance.

Vedder, whose experience with beriberi is long and intimate, believes the deficiency to be multiple because the "polished rice diet" is inadequate in vitamins A, D, E, pyridoxine, riboflavin, pantothenic acid and choline as well as thiamine. Yet Shimazono's reports are full of instances in which

But Grijns, his assistant, soon became convinced that something was lacking from polished rice which was curative. Grijns prepared extracts of the polishings which were curative. This signal step was taken as recently as 1901.

Pekelharing was quick to see the significance of these experiments and extended them by feeding mice a bread baked of casein, albumen, rice meal and salt mixture. If in addition the mice received only water "*they die of deficiency*. . . . But if milk is given instead of water they keep in health, notwithstanding that the quantities of albumen, lactose and fat they take with the milk are insignificant by comparison with those in the bread they consume."

This report as well as that of Stepp, who in 1909 demonstrated the inadequacy of pure fat in animal diets, received scant attention at the time. Hopkins classical experiments which identified "accessory factors of the diet" three years later received greater attention and with Funk's suc at about the same time in extracting an extremely potent antiberib fraction from rice pericarp and his monograph "The Vitamines" in whi the field of deficiency diseases was charted with great insight, thorough established the new knowledge in the attention of the scientific world.

Meanwhile the problem of the etiology of beriberi was being critically tested in the field. The next important step was taken by Frazer and Stanton who took 300 Javanese laborers into the jungle. Half were given polished rice, half less refined rice as the major item in their diets. Beriberi developed in the first group after three months whereupon the groups were reversed and the second group became sick. In other words, having controlled, environmental factors and under sanitary surroundings the seemingly insignificant change in food associated with feeding polished rice was found capable of producing or preventing beriberi. Four years later similar results were secured in Manila by Strong and Crowell.

It is twenty years since the opinion that beriberi is due to thiamine has been challenged. Yet various nutritionists consider it to be complicated by other dietary deficiencies. This opinion is based on the meagreness of the diets sometimes associated with beriberi, the failure of human volunteers fed a diet deficient only in thiamine to develop edema or cardiac dilatation, cardinal signs of the disease, and the slow recovery of nervous lesions under thiamine treatment. The problem doubtless deserves further investigation but certainly none of these objections is conclusive. Thus it must be remembered that whatever the dietary deficiencies may be, the nutrition of many patients appears to have been excellent. Neither anemia nor loss of body weight, both sensitive criteria of malnutrition, are necessarily associated with beriberi. And we fail to find evidence of symptoms of other deficiency diseases except in rare cases. The tongue, eyes and skin

to 1 mgm. of thiamine per day and that intakes of somewhat less than 0.5 mgm. have at times been preventive. These discrepancies are perhaps explained in part by the several factors known to influence human requirements in which case they would serve well to show the practical importance of such factors.

Williams and Mason set the critical level for adults at 0.5 mgm./1000 calories. This value was based on the behavior of human volunteers. Symptoms disappeared when the intake was increased to 1 mgm. which is estimated to be the consumption of individuals on a mixed diet of their own choosing (Elsom and Machella). It corresponds to the estimates of Stiebeling and Phipard, based on dietary surveys, the Committee on Nutrition of the League of Nations and of Lloyd and Orr whose calculations were based on a survey in Great Britain.

More recently there has been a tendency to increase the thiamine allowance and provision has been made to see that this is accomplished by enriching flour. Williams estimated in 1942 that the American diet averaged 0.8 mgm. per diem without enriched flour and 1.3 mgm. with it. This would just satisfy the minimal intake required to prevent "biochemical injury" as defined by Williams, Mason and Wilder and is somewhat less than the recommended daily intake of the Committee on Food and Nutrition of the National Research Council (0.6 mgm./1000 calories).

The Committee's "recommended tentative goal toward which to aim in planning practical dietaries" is as follows:

		mg. thiamine
Man	Moderately active (3000 calorie diet)	1.8
(70 kg.)	Very active (4500 calorie diet)	2.3
	Sedentary (2500 calorie diet)	1.5
Woman	Moderately active (2500 calorie diet)	1.5
(56 kg.)	Very active (3000 calorie diet)	1.8
	Sedentary (2100 calorie diet)	1.2
	Late pregnancy (2500 calorie diet)	1.8
	Lactation (3000 calorie diet)	2.3
Children	Under one year	0.4
	One to three years	0.6
	Four to six years	0.8
	Seven to nine years	1.0
	Ten to twelve years	1.2
	Girls thirteen to fifteen years	1.4
	Girls sixteen to twenty years	1.2
	Boys thirteen to fifteen years	1.6
	Boys sixteen to twenty years	2.0

The Committee believes that these values can be provided by a diet of natural foods and the experience of the United States Army proves this to be

beriberi occurred in severe form on institutional diets much more complete than those of Vedder's. Many of the items are unfamiliar and their value cannot be calculated, but fish, onion, vegetables, beans, sweet potato and other foods played a considerable part and must have supplied various vitamins. Rice was the largest item yet it should be noted that the mere substitution of barley, which has twelve times the thiamine content of polished rice, for 60 per cent of the rice sufficed to eliminate beriberi. procedure was official practice in the Japanese Army for many years. deed the evidence everywhere implicates thiamine. Takaki's success i case in point. The changes he made in the diet of the navy were such th would predominantly affect the thiamine content. It is reasonable t suspect that a further contributing factor to the great prevalence of beriber in Japan is the consumption of raw and undercooked fish which often contains an anti-thiamine enzyme (see Chastek paralysis). Both Shimazono and Hashimoto found quick and certain cure followed the injection of thiamine. It is therefore difficult to fasten on a single characteristic of beriberi which may be definitely associated with deficiency of factors other than thiamine.

In our own country conditions may well be different. Thiamine deficiency is frequently seen as a complication of other forms of avitaminosis, especially pellagra, but this evidence is remote from the problem of Oriental beriberi.

THE THIAMINE REQUIREMENT OF MAN

Reference has been made to the simple yet effective means which have been used in the past to prevent or cure beriberi. With the improvement in assay methods and increasing understanding of the problem precise measurements have become possible.

The pioneer work of this kind was that of Cowgill who developed a formula for calculating the thiamine requirement of various laboratory animals. This he tested in a variety of ways and then extended to man relying in this case not on experiments but the records of diets known to have resulted in beriberi and others which prevented or cured it. The resulting "prediction chart" serves well to identify the critical, beriberi producing level of thiamine intake in terms of calories and body weight. A simpler calculation can be made by Williams and Spies rule of thumb which is that the ratio between thiamine (expressed in Int. units) and non-fat calories in the diet shall be 0.25 (see page 70).

Whether because of differences in cooking or the nature of the food itself considerable discrepancy exists in the thiamine value of rations which have at one time or another resulted in epidemic beriberi. The analyses of Bak and Wright indicate that beriberi has occurred on diets containing from 0

excretion during pregnancy in 100 women. By the end of the period of gestation the average excretion was approximately half the initial value.

The influence of the metabolic rate on thiamine requirements is very definite. The exercising of dogs hastens the onset of symptoms of deficiency (Cowgill) and feeding thyroid accomplishes the same thing. Williams et al. have recently confirmed this observation in man and report that spontaneous thyrotoxicosis is associated with excessive thiamine loss in urine, feces and sweat. Excretion remains elevated despite low body stores.

Westenbrink found that pigeons developed symptoms quickly or very slowly depending on whether the basal diet was largely carbohydrate or fat. Arnold and Elvehjem confirmed this in rats. The thiamine requirement was reduced to one-third by adding fat in the amount of 50 per cent of the basal ration. There is a considerable difference depending on the length of the carbon chain of the fatty acids as was shown by Evans. The sparing effect of high protein diets is also considerable amounting to a third of the normal requirement.

If to these influences we add more obvious ones, failure to absorb thiamine because of many gastro-intestinal disturbances, destruction by alkali, bile and pancreatic juices (as shown by Melnick, Robinson and Field) and dietary habits, both good and bad, it seems evident that thiamine requirements in terms of the individual become a highly complicated problem.

The sparing effect of high protein and fat diets has been previously mentioned. High carbohydrate diets *exaggerate* the requirements. Alcohol is believed to operate in this manner and Jolliffe reported an instance in which the intravenous administration of large amounts of glucose precipitated cardiac symptoms having the characteristics of beriberi.

The influence of infectious diseases may be largely due to the resultant fever. In the Orient it is a predisposing influence of great importance. Schretzenmayr's view, based on a large experience in Canton, is illuminating. He considers most of the poor people and soldiers of China to be on the borderline of clinical deficiency. But relatively few develop beriberi without a precipitating cause which in most instances is an infectious disease, typhoid fever, malaria or pneumonia. Of his typhoid cases 70 per cent had beriberi and the others were patients who had received dietary supplements. Nearly two-thirds of the cases of intestinal parasitism developed symptoms of beriberi.

Meyer's views on this matter are especially interesting. He believes that thiamine deficiency appears under two distinct clinical syndromes: as a simple polyneuritis and as beriberi (polyneuritis, edema and cardiovascular dysfunction). In most cases of simple polyneuritis complicating factors, usually infectious, precede the nervous lesions. Polyneuritis, he writes, is not early beriberi but a special form of thiamine deficiency. In about half

In 1926 Fridericia gave the name "refection" to the spontaneous recovery of certain rats fed a thiamine deficient diet. Such animals continued to gain weight and thrive while their litter mates perished. In some cases the "immunity" was transmitted through several generations. The feces were distinctive; whitish in color and full of undigested starch. These observations were confirmed in several laboratories and were generally believed due to bacterial synthesis. As Kon described it "processes take place analogous to those normally obtaining in the bovine. These processes render the rat, like the bovine, independant of an exogenous supply of vitamin B." She found that raw potato starch could establish the conditions necessary for refection while cooked potato starch could not do so.

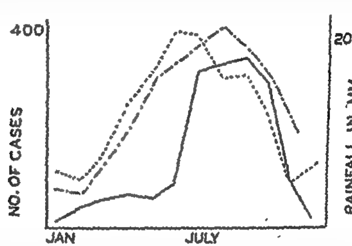

FIG. 35. Incidence of beriberi, rainfall and temperature as observed among Japanese soldiers. The number of cases is represented by the solid line, the rainfall in mm by the broken line. The dot-dash line indicates the temperature variation. (After Shimazono.)

The chief support for the theory that synthesis occurred was the demonstration of thiamine in the feces concurrent with clinical improvement.

Requirements are greatly modified by various other factors commonly met in clinical medicine. It is well known that diarrhea prevents adequate absorption. In dogs moderately severe diarrhea causes a loss of half the ingested supply (Dann and Cowgill). There is considerable evidence that persistent vomiting, as seen in hyperemesis gravidarum, causes severe depletion. *Temperature* has been known for many years to influence the occurrence of beriberi (Fig. 35) and Mills has shown that rats in an environment of 91°F. require twice as much thiamine as they do at a temperature of 65°F. Diuresis increases thiamine excretion and requirements (Cowgill et al.). The utilization of thiamine is interfered with by a variety of hepatic disorders. Other influences are well known. Pregnancy increases thiamine requirements. Van Coevorden charted the fall in urinary

excretion during pregnancy in 100 women. By the end of the period of gestation the average excretion was approximately half the initial value.

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the cases of polyneuritis without edema, heart function is quite normal. In the cases with edema 92 per cent of the cases have heart symptoms. The adrenalin test is negative in polyneuritis but present in typical fashion in beriberi.

It is more generally held at present that these differences are due to the degree of deficiency and the rapidity by which deficiency develops. Thus cardio-vascular manifestations have been ascribed to severe deficiency developing acutely or subsequent to prolonged, partial deficiency (Goodhart).

THE PATHOLOGIC ANATOMY OF THIAMINE DEFICIENCY

The morbid anatomy of thiamine deficiency is limited to degenerative changes in the nerves which are seldom demonstrated in acute deficiencies, certain rather characteristic changes in the heart and in some cases edema with effusions in the body cavities and signs of cardiac failure.

BERIBERI

The clinical differences between acute and chronic cases of beriberi have their counterpart in the appearance of the subjects on the autopsy table. The acute cases are often in good fat and show less degeneration of the nervous tissue than the chronic cases but the appearance of the heart is more striking since death is usually due to a cardiac attack. On the other hand the chronic cases seldom die unless complications intervene, and in such cases the appearance of the various organs is more the result of the tuberculosis, typhoid fever, or other infectious disease than of the beriberi itself.

Nervous System

The anatomic distribution of the nervous system lesions conforms with the clinical manifestations. The nerves supplying the lower extremities are most commonly affected but the cranial nerves and the vagus system are frequently deteriorated too. The peripheral portions of the nerves are first and most seriously altered. The cranial nerves above the 7th are rarely involved.

The nervous lesions are not recognizable macroscopically. Histologically they consist of a pan-neuritis which commences with vacuolar degeneration of the cells of Schwann. Later, fragmentation of the axis cylinders occurs and Wallerian degeneration may be demonstrated. Fat stains are suitable for studying the fatty degeneration. Collections of round cells appear in the nerve bundles.

The central nervous system is but slightly affected. The membranes of the brain are sometimes thickened and the ganglion cells in pons, medulla, and spinal cord are found degenerated. Since such changes are extremely

Fig. 1

Fig. 2

PLATE X. Fig. 1. Myelin degeneration in a peripheral nerve in a human case of polyneuritis. Fig. 2. Fatty degeneration of one of two branches of the vagus nerve in a woman whose death was associated with great dilatation of the right chambers of the heart. The right nerve is affected. No other organic changes found. The case is believed to have been one of beriberi. (Stained with Oil Red O.)

common in adults and may result from a wide variety of morbid processes they are of little or no value in arriving at a diagnosis. The ganglion cells show swelling of the cell bodies, peripheral displacement of the nuclei and partial or diffuse disappearance of the Nissl substance. Occasional fibers in the cord and brain show fatty degeneration. The cerebrum is otherwise unaffected.

Degenerative lesions are common in the muscles of foot and leg, although in some cases similar changes occur in the upper extremity, thighs, and diaphragm. The lesions are not characteristic histologically, consisting only of cloudy swelling, fatty degeneration, loss of striations and hyalin or waxy degeneration. In "wet" cases edema will be recognized in the muscle preparations and in the chronic, wasted cases an atrophy of the muscle fibers.

Heart

The acute cases reveal more illuminating changes in that the heart is involved. The most characteristic effect of the disease on this organ is its appearance in situ. Wenckebach fixed the hearts in his cases with formaldehyde solution before removing them, to preserve the shape and relative sizes of the various chambers.

Older observers have almost uniformly ascribed the cardiac manifestations to lesions of the vagus nerves. The only evidence for such an explanation has been the frequently described vagal lesions. But there is no evidence that vagal lesions ever produce the changes seen in beriberi, and good cause to doubt that vagal degeneration and cardiac symptoms go hand in hand. Indeed, the heart symptoms are seen in acute cases when the nerve lesions are scanty or underdeveloped.

The organ is hypertrophic and dilated. The average weight of the heart among Japanese patients has been given as 368 grams, while the maximum of normal hearts among the same population is 300 grams. Cardiac enlargement has been measured in 200 cases by Kobayashi using x-ray measurements. He found that the lung-heart quotient of Groedel is reduced from a normal value of 1.71 to between 1.19 to 1.43 in beriberi. The method was recommended as an aid in diagnosis. The enlargement of the heart is most pronounced in the right chambers and the left heart may be very small. The right auricle is huge, with paper thin wall through which the dark blood within may be seen. The walls are said to be friable and easily torn. The conus arteriosis is tremendously dilated, especially at the point of origin of the pulmonary artery. Wenckebach considers this to be pathognomic of this form of right sided dilatation. There may be petechiae at the auricular-ventricular juncture. The lungs and the left heart are not over-filled with blood. The impression is that the right chambers

are so feeble that they steadily enlarge as the site of least resistance. Epicardial petechiae and flecks of fibrin on the epicardium have been reported. Shimazono mentions fatty degeneration in the bundle of His. Weiss and Wilkins found hydropic degeneration of the muscle fibers, swollen collagen and perivascular edema and separation of the muscle bundles. These lesions were not constant.

Sambuc examined 52 cases of beriberi in Cochin-China and considered the appearance of the heart pathognomonic. The degree of dilatation, the location of the dilatation in the right side and the pericardial effusion constituted an unmistakable picture. Sambuc ascribed death in some cases to pressure from the effusion. Other cases died from rupture of the auricle.

The gross criteria of beriberi used by McLaughlin and Andrews are: (1) Dilatation and hypertrophy of the right heart; (2) congestion of the viscera; (3) anasarca, and (4) absence of other findings to account for death.

No satisfactory anatomical explanation of the heart lesions in beriberi exists. Microscopic examination shows nothing of importance. To circumvent the impossibility of examining heart muscle during the acute phases of the disease Wenckebach took small specimens of the calf muscles which he felt might be comparably affected. They appear normal in all respects but size. In the acute stage the fibers were larger than normal by actual measurement, but well marked and of usual structure. A second sample was taken from the same subject after treatment and showed the muscle fibers to be of normal dimensions. Wenckebach considers that the striated muscle of the calf had excessive bound fluid within its fibers and, by analogy, the heart muscle also. More recently Porto has described interfibrillar and interfascicular edema and perinuclear vacuolization in the muscle and conduction system cells of the hearts of dogs suffering from thiamine deficiency. He also observed some alterations in the appearance of the muscle striations and gross changes similar to those just mentioned as characteristic of human beriberi.

Anasarca, hydrothorax, and hydropericardium are very common in acute cases of beriberi, being seen also in some chronic cases. The presence of transudate in these cavities, as well as the degree of dilatation of the heart depend in large measure on the condition of the patient before death. Persons who have been active are more liable to show such changes, while bed rest permits compensation and permits the heart to return to more nearly normal dimensions, and for fluid to be resorbed.

On the basis of such observations the fluid in "wet" cases of beriberi has been considered to be purely a transudate. The edema of the lungs and liver (in which cases congestion and central necrosis in the latter also occur) have been interpreted similarly. However most cases of beriberi have been judged by rather primitive criteria and it is not surprising to find that recent

studies have denied the transudate theory. Wenckebach's views imply another mechanism. Eppinger has commented on Wenckebach's material and given his interpretation of the lesions in the liver specimens collected by Wenckebach. He considers the fluid to be the product of a serous inflammation and found histological lesions of serous hepatitis in all cases. These changes included the widening of Disse's spaces (the controversial lymphatics said to lie between the capillaries and liver cells), lymphatic stasis and periportal edema. Edema was also present in the bed of the gall bladder. Toll has found a thickened gall bladder wall constantly present in beriberi.

It should be apparent that this view, implying the presence of an irritant, is much more in harmony with the work of Peters than the older conception and that while it still lacks the support of other investigators offers some hope that further advances in the clarification of the pathogenesis of beriberi may be expected from anatomic studies.

Eppinger's views are not incompatible with the observation previously noted that active cases present more free fluid in their tissues and serous cavities. The relationship between vitamin B_1 requirement and metabolic rate suggests the deficiency may be more aggravated in active patients and the production of irritants due to faulty metabolism increased.

The pancreas is at times shrunken and even slightly cirrhotic. The islands of Langerhans are either not affected or are hypertrophic in conformity with the altered carbohydrate metabolism seen in beriberi.

The adrenals are sometimes enlarged due to medullary hypertrophy. A slight lymphocytic infiltration is frequently mentioned. Nagayo and Katsunuma reported hypertrophy of the hypophysis and thyroid glands and Katsunuma found lymphocytic and plasma cell infiltration in the pars intermedia of the hypophysis in all his cases.

The lymphatic apparatus is not reduced in acute cases. Atrophy of these tissues is generally believed due to inanition and not beriberi. Nagayo, indeed, found cases with status thymico-lymphaticus and considered what part this may have played in their sudden exodus.

The cervical and splanchnic ganglia and plexus of Auerbach have all been reported as showing degenerative changes. The larger portions of the vagus have all been found showing changes similar to those in other nerves. Changes have been reported by many pathologists in the nerve endings, both motor and sensory but these are difficult to study. In infants, Honda found lesions of the laryngeal nerve but not of those in the extremities. This is probably explainable on the basis of usage.

Vedder has written that "although the symptomsof beriberi have been attributed in the past chiefly to peripheral neuritis, the condition is not a simple peripheral neuritis but is a degeneration of the entire nervous sys-

tem." It is true that some cases of peripheral neuropathy extend along the posterior nerve roots and that demyelinating lesions in a few instances are demonstrable in the posterior columns, chiefly the column of Goll. Extreme inanition sometimes also results in axonal degeneration of the anterior horn neurones (Zimmerman). The changes which have been described in the brain are of minor degree and doubtful etiology. They are of no diagnostic value as Broder pointed out. Kihn and Davidson's studies led to a similar conclusion.

It may be seen from the above that the post mortem studies of beriberi have contributed little as yet to our understanding of the pathogenesis of the disease. The diagnosis of beriberi post mortem must depend on the association of a rather characteristic cardiac dilatation and hypertrophy without evidence of organic cause and degeneration of the peripheral nerves or, in the chronic cases, a disseminated, irregularly distributed myelin degeneration with or without wasting or edema. To these rather unsatisfactory criteria may be added other of the lesions described.

It is to be ardently hoped that future studies will sharpen our perception of the essential changes in this interesting disease to such a degree that we can identify not only the well developed case but those incomplete forms which now pass unrecognized.

EXPERIMENTAL BERIBERI

Eijkman's experiments were based on fowl fed only polished rice. In the third week of such a diet the animals become paralyzed and the head is retracted. Within a few days they die unless thiamine is given in which case complete recovery occurs within a few hours. Two things stand out from this story. The diet is obviously deficient in many elements besides thiamine and the duration is much too brief to permit the development of organic changes such as characterize beriberi. McCarrison recognized this artificiality of fowl polyneuritis twenty years ago and modified the diets by supplying small amounts of vitamin. His animals thereupon developed lesions and behavior more like the natural disease, including enlargement of the heart.

The same thing occurs in rats. The usual laboratory diet, composed of casein, starch, salt mixture, cod liver oil and butter fat and supplemented with the other [illegible] within 3 weeks and later nerv[illegible]d stiff and the animals often move in a circle. When held by the tail the rats revolve in a characteristic "circus movement." These effects are due more to inanition than thiamine deficiency and the anatomical changes bear this out. True paralysis can only be produced by feeding a thiamine low rather than completely deficient diet.

Lesions of the Nerves

A recent study by Prickett, Salmon and Schrader, in which the discrepancies of earlier workers is reviewed, seems to demonstrate that under comparable conditions lesions in the nervous system may be produced in rats identical to those which occur in man. The examinations were made largely with Nicol prisms. "At autopsy the peripheral nerves of a few animals that had shown severe symptoms were observed to have localized enlargements along their course." The histologic changes included swelling of the sheath and axis cylinder. The nodes of Ranvier were enlarged and dumb-bell shaped. In the severe cases considerable isotropic material was present and the axis cylinders roughened and irregular. Yet the occurrence and severity of the lesions was to a considerable degree unpredictable as other workers had found. The greatest success in correlating nervous lesions with clinical behavior appears in Swank's study of thiamine deficiency in pigeons. Swank found opisthotonus an expression of acute deficiency or a terminal manifestation in partially deficient birds. In the former case it was not associated with nerve lesions. In the partially deficient animals however nerve lesions were quite uniform and were constant if weakness of the legs had been noted clinically. The number of degenerate fibers in the sciatic nerve corresponded to the degree of paralysis. Regeneration was followed in treated animals.

In conformity with earlier reports Swank found the longest nerves to degenerate first and degeneration to commence in the distant parts of the fiber. Degeneration was associated with chromatolysis and eccentrically located nuclei in the dorsal ganglia. In the spinal cord degeneration was observed mainly in the ventral funiculus and spino-cerebellar tracts. Swank refers to unpublished observations which indicate that starvation induces only sheath changes (fatty droplets) and that evidence of nerve fiber deterioration is essential to a diagnosis of thiamine deficiency. His report is also interesting in that many of his pigeons died with evidence of congestive heart failure (congestion of the lungs and liver) and some of these showed focal necroses of the myocardium. Hydropic degeneration was never seen.

No mention is made in most reports of the condition of the terminal portions of the nerves. Tsunoda and Kura, however, describe changes in the ends of both sensory and motor nerves which were observed to respond within 5 hours to specific therapy. Woolard reported similar lesions. Woolard described the lesions in the motor terminae as swelling with bulbous enlargements and loss of detail. These were the first manifestations of deficiency and were followed by changes in (a) the sheaths and (b) the axis-cylinders.

The evidence is therefore very convincing that thiamine depletion is the

cause of the nervous lesions. Swank and Prados have furnished histologic evidence that the signs of severe thiamine deficiency in the pigeon and rat are associated with nervous lesions. The pigeon characteristically shows opisthotonus and rotation of the head. The rat (Church) passes through stages of hypotonia followed by ataxia, disturbed equilibrium (sometimes with nystagmus) and finally excitement. These could be explained by the release of central vestibular structures from labyrinthine control. Swank and Prados found degeneration of the sensory epithelia of the labyrinth and the central terminations of the vestibular neurones. From an anatomic point of view therefore there seems to be only one conclusion compatible with the evidence and that conclusion must be that thiamine deficiency promptly affects the finer structures of nerves, that degeneration extends along the neurones, that recovery under thiamine treatment is rapid or slow

TABLE XXX

Frequency of Certain Lesions in Rats Fed a Diet Containing Slightly Inadequate Amounts of Vitamin B_1 throughout their Lives

(Based on the studies of Drummond, Baker, Wright, Marrian and Singer)

	ADEQUATE DIET	DEFICIENT DIET
	per cent	*per cent*
Pneumonia (all kinds)	9.5	9.3
Emphysema	6.6	9.0
Gastric distension	2.2	11.0
Erosions	5.3	16 5
Chronic peptic ulcers	7.4	20.7
Hairballs	2.6	9.5
Adrenal enlargement	4.9	16.4

depending on the *extent* of the nervous degeneration. This is, of course, true of all degenerative lesions of the nervous system.

Whether the lesions are simply due to inadequate carbohydrate oxidation or to the accumulations of toxic metabolites is not known. It has been suggested that methyl glyoxyl is responsible. Williams has called attention to the evidence that the milk of women suffering from beriberi contains a toxic substance. Similar toxins have been demonstrated in the blood, urine and tissues of experimental cases of the disease. Such an explanation of the pathogenesis of beriberi, i.e. deficiency operating through a toxin, would explain many features of the disease.

Gastro-intestinal System

There has been a steady growth in our knowledge of the effects of thiamine deficiency on the gastro-intestinal system. The functional disturb-

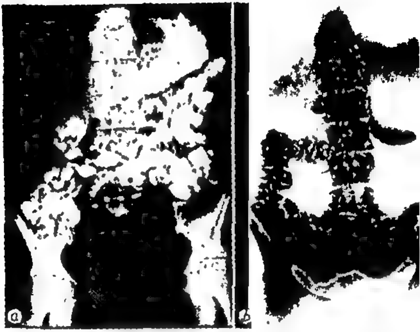

PLATE XI Effect of experimental thiamine deficiency on gastrointesti
Roentgenograms made three hours after a barium meal Roentgeno
taken after approximately three months of thiamine depletion, roent
after 15 days on the same diet but during which supplements of thiamine
ride had been given (Originally reproduced in Arch Int Med, 66 791
produced by permission of Dr Russell M Wilder)

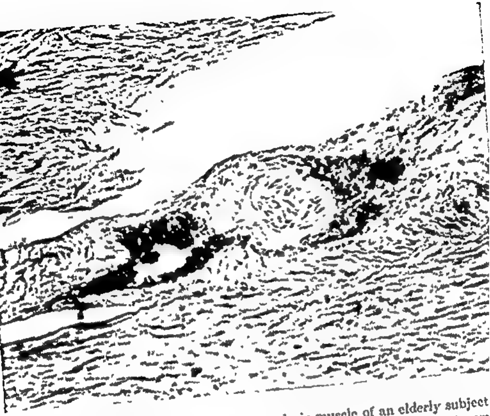

PLATE XII. A diseased ganglion in the pyloric muscle of an elderly subject
had pyloric hypertrophy and whose chief complaint had been persistent vom
and distension. The nerve cells are degenerate and the ganglion is largely rep
by scar. It is surrounded by hemorrhages. The lesion may be due to this
deficiency.

ances are well recognized. Loss of appetite was firmly established by the work of Cowgill, Deuel, Plummer and Messer demonstrating gastric atony by means of a balloon inserted through a gastric fistula. Cowgill and Gilman showed that gastric acid (in dogs) was reduced or absent. Atony of the intestinal tract as well as the stomach was demonstrated by Rowlands many years ago and degeneration of Auerbach's plexus was reported by McCarrison. Sparks and Collins more recently have re-examined the atony effect of thiamine deficiency. Their method was to actually measure the capacity of the large bowel. The bowel was first cleansed and then filled with a radiographically opaque fluid to the ileocaecal valve. In the normal rat this required from 3 to 4.4 ml. Using rats on a thiamine deficient diet the capacity was greatly increased in 70 per cent of the animals. In most of these the capacity was doubled. The effect was believed due to thiamine alone.

Gastric erosions and ulcers occur in thiamine deficient rats. McCarrison first observed this. In the experiments of Dalldorf and Kellogg the incidence was high and the chronicity of the ulcers seemed related to the duration of the deficiency. Partial deficiency was necessary, the rats being maintained near the level of symptoms for some months. Recently Thatcher, Sure and Lee, in a comprehensive study of the histopathology of the avitaminoses state: "Perhaps the most significant result in the investigation is the finding of gastric ulcers as a result of the specific influence of a deficiency of vitamin B_1." Inanition was controlled by holding the controls to the same intake as the experimental animals. Other B factors were supplied. Of 8 rats 5 had ulcers.

Simpson failed to find ulcers. Adult animals were used. The experiments extended over 4 months. Ulcers have been found by Schiödt, Drummond et al. and Howes and Vivier. The latter found that young animals are more likely to develop ulcers than older ones. Evans, Carlson and Green found ulcers in the stomachs of foxes suffering from thiamine deficiency (Chastek paralysis). The incidence was from 10 to 30 per cent, much greater than in animals which died from other causes.

Drummond's experiments are very conclusive. More than 1000 rats were used, the deficiency was very slight but prolonged throughout the life of the animals. Ulcers and erosions were common in the deficient animals. Most of these lesions are simple, punched out erosions. However, erosions are believed to be the first stage in the formation of chronic peptic ulcer and in Dalldorf and Kellogg's rats chronic, indurated lesions were occasionally found. We have found no record of gastric ulcers in human cases of thiamine deficiency. The mechanism by which they occur in the rat is obscure. Mid-brain lesions are often associated with gastric ulceration in man and the dog and the "chemical lesion" in experimental beriberi involves this part of

the brain. The production of ulcer by vagotomy, recently reviewed and studied by Beazell and Ivy, may afford a clue. Considerable evidence also exists to show that the terminal nerve structures in the gastric wall are diseased in peptic ulcer. Stöhr's studies have confirmed this and shown that these minute lesions are widely distributed in the gastric wall. The cells of both Auerbach's and Meissner's plexi are altered, an observation made in experimental thiamine deficiency by McCarrison.

A degree of deafness was demonstrated in rats and chicks on a thiamine deficient diet by Selfridge and Maurer and Tsai, by means of maze tests, showed that partial depletion stigmatized the learning ability of their animals. In the latter experiments the deficiency was multiple. It would appear that the entire nervous system suffers from lack of thiamine and that the occurrence of lesions is determined by secondary and often local factors. This is in harmony with the lesions in man to which reference has already been made.

Glands of Internal Secretion

The adrenal glands are hypertrophic in experimental thiamine deficiency and Ogata found lesions in the associated ganglia as well. During the active phase of the deficiency the cortical cells show considerable mitotic division. The islets of Langerhans are also hypertrophic (Wolbach, Ogata, Ueno). Kihn found this difference small but constant in rats while in pigeons and fowl the hypertrophy more than doubled the normal weight. The thymus, pituitary, thyroid, spleen and liver are reported to be atrophic. Whether this is due to inanition is unknown. Earlier reports of atrophy of the seminiferous tubules and disappearance of sperm cells are believed by Evans and Bishop to be due to other deficiencies. However, loss of libido occurs in male animals and prompt suppression of follicular function in the ovaries of females. Evans and Bishop showed this to be an early effect of the deficiency. The phenomenon was extensively studied by Ueno who was able to maintain normal estrus by administering follicular and anterior pituitary hormones.

Heart

In the early experiments with pigeons and rats cardiac hypertrophy and dilatation were never seen. McCarrison first showed that they could be produced in pigeons but his diets were "natural" and his success did not settle the problem. We now know that McCarrison succeeded because he produced a chronic stage of partial deficiency. Under these conditions all tested animals eventually do develop cardiac changes. Wintrobe has lately described typical cardiac lesions in pigs.

A comprehensive study of the relationship of thiamine deficiency and the

heart in pigeons was reported by Swank and Bessey. They succeeded in producing congestive heart failure, tachycardia, changes in the EKG and focal necrosis of the myocardium by slowly inducing deficiency while maintaining food intake by forced feedings. Both thiamine and cocarboxylase were shown to afford complete cure. They suggested that "the tachycardia is due to vasodilatation, which is caused by the local accumulation of intermediate products of carbohydrate metabolism. This also facilitates transudation of fluid from blood vessels to form hydropericardium and other evidences of cardiac failure. In addition thiamine deficiency impairs the function of the heart, increases the tendency to extravascular fluid collections and results in terminal cardiac standstill."

Ashburn and Lowry slowly depleted 57 rats of thiamine and then maintained them for from 33 to 145 days by giving a dose of thiamine whenever symptoms of acute deficiency developed. In all but 10 the auricles showed necrosis of muscle fibers or fibrosis and other signs of repair. Only 7 had lesions of the ventricles and these were small. The auricles were dilated at death and a number of animals were found to have increased amounts of fluid in the body cavities. Inanition was controlled.

Central Nervous System

Small hemorrhagic lesions in the pons, medulla and cerebellum of deficient pigeons and rats have been recognized since 1934 when Prickett described them. It is now believed that they are the experimental counterpart of Wernicke's disease as first suggested by Zimmerman and by Alexander. The lesion consists of dilatation (varicosities) of the cerebral vessels near the 4th ventricle. A considerable degree of hyperplasia of the vascular endothelium may be seen although Alexander characterizes the lesion as primarily an angiodegeneration. He believes the subacute parenchymal necrosis is secondary. Swank and Prados consider the hemorrhages due to degenerative changes in the neurones adjacent to the vessels. These lesions formed a conspicuous finding in Chastek paralysis of foxes and greatly contributed to its recognition as a form of thiamine deficiency.

Chastek Paralysis

This instructive and spontaneous form of thiamine deficiency occurs on fox farms. The symptoms appear after a period of 7 to 10 days of anorexia and consist of rapidly increasing weakness, ataxia and spastic paralysis. Death follows in 2 to 3 days, at times preceded by convulsions.

Loss of appetite is the first symptom and is followed by slowly developing spastic paralysis At first the animal runs with short, stiff jumps. It tends to lie down much of the time. Later stiffness and incoordination are very

extreme. Yet the animals remain *completely alert*. If death does n occur quickly the paralysis becomes complete and the animals lie in a sti ened condition with their heads drawn back. Some can be turned ov like a board. Green called it Chastek paralysis because he first saw it the Chastek farm in Minnesota. Repeated efforts to demonstrate an i fectious agent yielded negative results and it was noted that all outbrea were associated with the feeding of large amounts of fish or fish produc This diet was followed by symptoms after an interval of 3 to 6 week Dietary experiments confirmed the opinion that the disease was not due a toxic substance in the fish but to thiamine deficiency. Thus two grou of foxes on the same ration (in which carp represented 20 per cent b weight), one which received 25 mgm. thiamine chloride per diem as a su plement remained well while the controls developed characteristic sym toms after three weeks. Green and Evans, on the basis of dietary an anatomical studies, not only identified the disorder as due to thiamine d ficiency but postulated that the fish induced the deficiency. Since th lesions were vascular in nature, dilatation and varicosities of the cerebr vessels in the region of the 4th ventricle, they further characterized it as th equivalent of Wernicke's disease. They associated the anti-thiamin action with the viscera, head and skeletal portions of carp. The muscle harmless. The factor which destroys thiamine is labile to heat and dryin at room temperature and extractable with 5 per cent alcohol. Deutsc and Hasler found the enzyme in various fresh water fish but not in sa water ones. Ender and Helgebostad described the disease in mink an ferrets as well as foxes. They called it beriberi.

The conditions under which Chastek paralysis occurs are very exactin The fish must be raw and ground or in other ways intimately mixed with th rest of the food.

The very young die before lesions develop sufficiently to be recognized The heart shows intracellular edema and degeneration. Congestion, hem orrhages and necrosis are common in the liver.

It is interesting to note that Zimmerman has found the Wernicke lesions in dogs only after prolonged, chronic deficiency whereas they appear in foxes after 40 days depletion. He postulated that the explanation may be a species difference.

SYMPTOMS OF BERIBERI

It is customary to consider the nervous signs of beriberi the first to appear. This is probably not true. Significantly, Shimazono, in characterizing the disease, lists the three major features in the following order: cardiovascular symptoms, edema and neuritis. The usual sequence is mild cardiac symptoms with slight edema and dyspepsia followed by nervous symptoms.

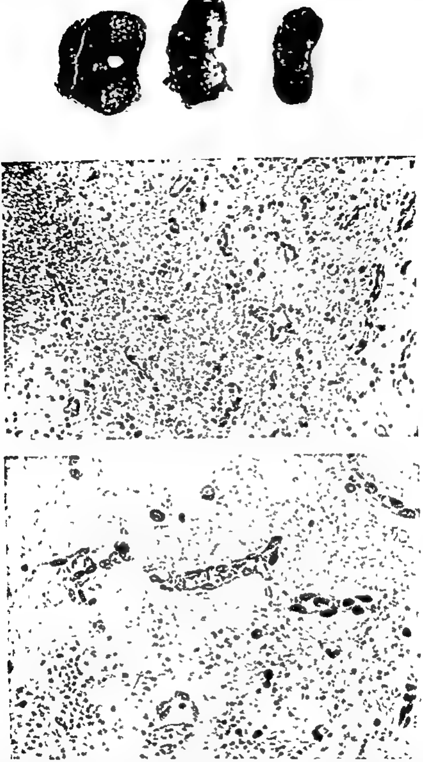

PLATE XIII Chastek paralysis or Wernicke's disease in the fox The upper figure shows the hemorrhagic lesions at three levels of the brain stem. The lower figures *show the minute structure , Note the vascular dilatation in the lower figure.* (Courtesy of Dr Charles Evans Originally reproduced in the American Journal of Pathology, vol. 18, page 91.)

Circulatory Manifestations

In the early stages of the disease palpitation is present and dyspnea occurs with great effort. The heart action is increased, the apex extends beyond the mid-line and epigastric pulsation is very common. The outline of the heart is enlarged and rounded. Miura states that enlargement is first to the right, then to the left and upwards. During recovery the right margin is the first to return to normal. The heart sounds are exaggerated, especially the second pulmonic one and the pulse is rapid. In severe cases the rate exceeds 100 p.m. The rate is very labile and responds to the slightest exertion. The pulse wave is large and full. A rate of 120 to 130 in acute cases, or chronic ones which have become acute, is a warning signal. The rate slows rapidly under treatment and may become abnormally slow during convalescence. Systolic pressure is not affected but the diastolic pressure is almost constantly low. A murmur may be heard over the veins, particularly the femoral one. Miura heard this cruraltone when standing at the bedside of a patient.

Wenckebach described in detail the cardiac symptoms as he saw them in Java. He felt the following to be particularly helpful in establishing a diagnosis:

1. Enlargement of the heart by percussion, auscultation and x-ray examination.
2. The presence of murmurs, chiefly systolic but also presystolic, with a resonant first sound. The murmurs are disproportionately increased by exercise.
3. Visible and palpable throbbing pulsations over the heart, best felt just to the left of the sternum.
4. Bounding pulse and thrill over the great arteries
5. Over-distended neck and arm veins and, *without exception*, a painful, swollen liver. In the most severe cases liver pulsation.
6. The electro-cardiogram remains normal throughout, though a slight shortening of the auricular interval has been reported.

Nervous Symptoms

The neuropathy of epidemic beriberi is strictly peripheral. The psyche remains clear. We have found no record of psychosis of the Korsakoff type in the Japanese literature. From the beginning of the disease the patients complain of disturbed sensation in feet and legs, sometimes in finger tips as well. This disturbed sensation is described as though their skin was covered with a sheet of paper. Later pain and weakness occur in the legs, walking is tiring and cramps may occur in the thighs or calves at night.

The sensory disturbances begin in the feet and legs but both the finger tips and a zone about the mouth may be affected. Miura states that a fourth area of hyperaesthesia is the skin of the lower abdomen below the umbilicus. The areas of hyperaesthesia do not correspond to the

distribution of particular nerves and their margins are indefinite. The sole of the foot is much less affected than the dorsal surface and the perineum is very rarely involved. However in severe, chronic cases the forehead and skin about the eyes may be affected. In these cases the sensory changes in the lower extremities show considerable anaesthesia to touch, not to pain. As the sensory changes develop epicritic sensation is first affected, then temperature and pain sense. Vibratory sensation is also disturbed. It is said to be the last to return with recovery. Position sense is destroyed to a slight degree.

The motor disturbances also begin in the legs and usually are limited to the lower extremities. All degrees of change from weakness to paralysis have been noted. Toe drop is present with motor lesions; the gait becomes broad based. In such severe cases the hands may be weak and Miura described a peculiar position which the hand assumes in which it hangs limply with but slight flexion in the phalangeal and metacarpal-phalangeal joints. In these cases the diaphragm and recurrent nerve and sensory branch of the 5th cervical nerves are paralyzed. Head and neck movements are not limited. Laryngeal paralysis, edema and congestion quite frequently occur. Facial weakness is bilateral and consists of poor closure of the mouth, slight ptosis of eyelids and limited extension of the tongue. This is an uncommon sign. Moore and Shattuck are of the opinion that the case of paralysis described by Landry and from which the term Landry's paralysis has arisen was a case of beriberi. Shattuck feels that an important group of ascending paralyses are due to thiamine deficiency.

The motor disturbances, like the sensory ones, are frequently more severe on one side than the other. If both motor and sensory changes are unequally present they are almost invariably most severe on the same side. The reflexes in the lower extremity are first over-active and then gradually become weaker and weaker. The sphincters are not involved. Abdominal muscle weakness may cause difficulty in urination.

Little is found in the eyes. Certain writers have reported cases with night blindness and amblyopia due to central scotoma. Taste and hearing are unaffected.

Pain on pressure is commonly met with in the muscles of the calves early in the disease. This is an important diagnostic point. The nerves are not tender to pressure.

Edema

It is said that experienced physicians, in countries where beriberi is common, often suspect the disease from the pallor of a patient and a slight degree of edema of the legs. The edema is not related to the cardiac function or to disturbances in the kidneys but probably is related to the vascular

function. In the mildest cases it is precipitated by exertion, in more advanced cases it becomes permanent. If the patient be bed ridden it occurs in the back and shoulders. It is often associated with slight pleural effusion but the latter may occur without edema. No satisfactory explanation has been offered for the edema. It is present to some degree in most cases but in only a few does it become prominent. The suggestion has been made that it represents a protein deficiency but proof is lacking and clinical facts do not support this view.

Schretzenmayr has made an interesting observation regarding the edema of beriberi. It is, he writes, never deforming. If the face is involved the features are not distorted as is true of nephritic edema.

Of the various forms of beriberi, the "wet" and "dry," the acute and chronic, more will be said later on. It is more important here to note that in beriberi countries it has been well recognized that a stage of incipient beriberi may occur, so mild the patient may hardly be called sick at all and that a latent form of the disease marked by residual signs is common after recovery. Thus, in examining a group of factory workers, Shimazono observed many with low diastolic blood pressure. More careful examination revealed mild beriberi in all of these men. The observation is all the more striking because such individuals, as indeed most patients with uncomplicated mild attacks, are in good fat and look to be well nourished. Indeed, most cases keep their weight during the attack, the exceptions being the acute cases with vomiting and the edematous cases which become "dry."

Fever occurs only in the acute form of beriberi and in these cases for but a few days before death. The fever in other cases is always due to another, coexisting disease.

The acute cases of beriberi may show a slight hyperglycemia, in other cases the sugar, calcium, albumin-globulin ratio are normal. The blood morphology is not affected. The anemia seen in some cases is explainable on other grounds, parasitism, etc. Shimazono believed that eosinophilia was a rather constant feature of the blood, probably due to nerve damage and Nakamura reported the platelets increased in number. The urine is not qualitatively changed but is suppressed in many cases. Sudden diuresis of large amounts of urine, two to four liters, is sometimes observed during recovery.

A sense of pressure or pain in the stomach is complained of by many patients as well as poor appetite and eructations. Scheube found gastric symptoms in 25 per cent of his cases. Most writers consider them commoner. They are a feature of the early stages of the disease. The acute form, Shoshin, is characterized by vomiting, first of food, later of gastric secretions. Vomiting occurs as a result of the slightest movement or effort. Constipation is frequent, diarrhea uncommon.

Kitamura and Shimazono followed the gastric secretion in many cases and found it reduced from the earliest stages of the disease and developing into achlorhydria. This process could be reversed by treatment. Goodhart and Sinclair studied 100 individuals and found a definite association between the blood cocarboxylase and gastric acidity.

The vital capacity is reduced in beriberi. Weakness of the diaphragm and abdominal muscles is largely responsible. Fukui demonstrated this by inserting a rubber balloon into the rectum of patients and measuring the changes in pressure. No respiratory pulsation occurred in the advanced cases of beriberi. The severe cases of cardiac failure are complicated by pulmonary edema.

Beriberi in Infancy

Beriberi in infancy is an interesting expression of the deficiency first recognized clinically by Hirota fifty years ago. It affects breast fed infants and only those whose mothers have beriberi. Not all beriberi women have beriberi infants. In some cases the mother is but slightly affected by the disease while the infant is severely afflicted. Shimazono states that those cases which he has investigated, in which the infant was sick and the mother appeared normal, all showed some physical signs of beriberi in the woman.

The cases occur in the summer and early fall, nearly twice as frequently in male as female babies and the peak incidence is in the second month of life. The cardiac signs are pronounced but the earliest and most important symptom is loss of appetite and vomiting. Ohta found that these little patients had a small gastric capacity, could take but a small amount of milk at one time. Urine is scanty, face pallid, pulse and respiration labile and rapid. The cranial nerves are much more commonly affected than in adult cases.

Loss of voice—aphonia—is also common among infants and is said to be highly characteristic. The child can only moan or whine in a plaintive fashion and the "beriberi cry" has made so distinct an impression on clinicians that the diagnosis is often made on the basis of this one symptom. Eighty-five per cent of Ohta's patients were hoarse. Laryngoscopic examination showed paralysis of a vocal cord in each case, the left cord being more frequently involved than the right. Drooping of an eyelid—ptosis—was also common. Ohta observed that the slightest infections such as common colds resulted in exacerbations of the beriberi. Some physicians have attributed the laryngeal paralysis to pressure by the dilated right auricle.

Diagnosis is usually made only after the onset of acute symptoms, the most characteristic being sudden paroxysms of pain associated with rigidity of the body which is held tense and straight. No true convulsions occur.

The face is cyanosed, the neck veins engorged, the pulse small and rapid. The pulse rate rapidly increases on movement, being very labile. The infant cries in a low plaintive voice and has repeated attacks of similar nature until death occurs, often within twelve to twenty-four hours, unless treatment is effective.

In forty-eight of Ohta's cases special studies were made of the gastrointestinal function (Ohta and Izumita). The group as a whole showed reduced total acidity and free hydrochloric acid although the values in the individual cases were extremely variable. The daily fluctuation in acidity was abnormally pronounced and this fluctuation, as well as the amount of acid, returned to normal under the influence of vitamin therapy. However the response is not always prompt. The authors divided their cases into four groups, depending on the type of gastric dysfunction present. The majority of their cases fell into a group characterized by anacidity or hypoacidity. In this group the reduced acidity was found to persist for from two to three months after the beginning of the treatment.

No delay in the passage of test meals was observed by these authors who found normal values for the emptying time of stomach, small bowel and colon.

Vedder has described an uncommon form of the disease in infants which runs a protracted course and is marked by obstinate constipation, vomiting, usually occurring every day at about the same time but unrelated to meals; restlessness at night, enlarged heart and developing pallor and weakness. Bray's experience seems to have been somewhat similar. He noted on Pleasant Island, in the Pacific, that coincidental with a change in the native diet, the substitution of canned foods for the native "toddy," the infant mortality increased alarmingly. Breast fed infants were most affected. The symptoms were vomiting, distension, irritability, intense cyanosis and convulsions. Death occurred suddenly. At necropsy the right heart was distended. Some of the infants had symptoms of meningitis or pneumonia but bacteriological examination was negative. The duration of the disease was very brief, 12 to 24 hours, and vitamin therapy successful. Bray described a different clinical syndrome in older children which he also described to thiamine deficiency. The symptoms were loss of weight and appetite, subnormal temperatures and susceptibility to infectious diseases such as bronchitis, otitis media and pharyngitis. The fatal cases also showed right sided heart failure and degenerative changes in the nerves.

Ship Beriberi

Atypical forms of beriberi encountered in our own country are discussed later on but one type, ship beriberi, is best considered in connection with the epidemic disease since it is no longer a medical problem. The disease has

not been well studied because it has occurred almost entirely on board sailing ships which seldom carried a medical officer. The nervous symptoms of beriberi appear in characteristic manner as well as weakness, constipation and loss of appetite but other symptoms are common which are not the result of thiamine deficiency. Thus skin petechiae and hemorrhagic gingivitis are often reported which are due to scurvy and night blindness which is due to vitamin A deficiency. Protein deficiency edema has undoubtedly been present in some instances as well.

Predisposing Factors

Six predisposing factors are listed in the Japanese literature:

1. Temperature. Cases are commonest in the warm months. This seems independent of fluctuations in the thiamine content of the food.
2. Humidity. Both the military and industrial records show that high humidity predisposes individuals to beriberi.
3. Age. It is a disease of young people, the majority of the patients being between 15 and 30 years of age.
4. Sex. Males are affected 2 or 3 times as often as females.
5. Robustness. Active individuals seem to be predisposed to beriberi. Scheube noticed this in 1894. Army records show that forced marches precipitate attacks Shimazono observed 8 cases among girls working in a spinning plant. They sat at their machines and worked almost entirely with their left hands. All 8 had hyperaesthesia of that part of the body and no other.
6. Physiological strain. Pregnancy, lactation and infectious diseases may precipitate beriberi. McKenzie ascribes one case to hookworm infestation and the loss of blood due to the parasites.

Complications are frequent, particularly infectious diseases. If this happens the cardiovascular disturbance is greatly intensified. Cases of chronic infectious disease are liable to develop beriberi, as has been mentioned, and in these circumstances the nervous disturbances are especially severe and very resistant to treatment. Severe paralyses are common in the beriberi of pregnancy also.

The most serious complication is the development of Shoshin. Young adults constitute most of the cases. Great weakness, thirst and dyspnea occur with loss of appetite and vomiting. The pulse rate mounts to 120, the respiratory rate 30 p.m. The urine is steadily reduced in amount, precordial pain may appear, the patient is most anxious and restless. Usually these cases show no nervous lesions other than areas of hyperaesthesia in the legs. They run a stormy course and many die within 3 days. Recovery is very rapid under treatment.

Vedder examined patients suspected of latent beriberi by testing for calf tenderness and searching for areas of anaesthesia in the lower legs by means of pin pricks. He then had the suspects squat on their heels. This position

is very painful in beriberi and patients frequently are unable to rise without pulling themselves up with their hands.

If a dependable dietary history is available presumptive evidence of beriberi may be secured by calculating the adequacy of the diet by Cowgill's method. The best diagnostic aid at present seems to be the therapeutic test. In acute cases improvement may occur suddenly and dramatically and even the chronic and atypical cases respond promptly to large doses of thiamine.

Certain aids in clinical diagnosis are listed by Shimazono. Nerve tenderness, generalized anaesthesia, severe ataxia, marked difference in involvement in two sides, cranial nerve involvement, disturbed reflexes, foot and patellar clonus and involvement of bladder and rectal sphincters are all absent in beriberi and serve to distinguish it from other diseases.

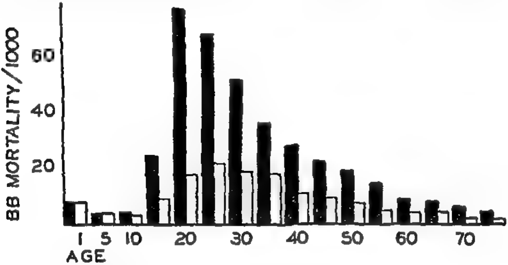

FIG 36. Beriberi mortality in different age-groups. The solid columns represent deaths among males, the hollow columns females. (After Shimazono.)

The prognosis in beriberi is now good. Uncomplicated cases seldom die unless of Shoshin attacks which are always a serious matter. In Japan the mortality rate is 2 to 4 per cent. In Malay Castellani and Chalmers report the mortality to have been 20 per cent. Early diagnosis and prompt medical care are capable of controlling beriberi in all but the most unfavorable cases.

DIAGNOSIS

Beriberi is considered relatively easily recognized clinically. The combination of neuritis, cardiac symptoms and edema are unique. When either of these manifestations occurs separately the problem is very different.

The characteristic distribution, spread and natural history of beriberi neuropathy is fairly characteristic. Differentiation from other forms of peripheral neuritis may be aided by Table XXXI. The cardiac signs cannot be identified without a history of conditions predisposing to thia-

TABLE XXXI

Comparison of Clinical Characteristics of Various Forms of Neuritis

	NUTRITIONAL	INFECTIOUS	DIPHTHERITIC	"JAKE" PARALYSIS	LEAD NEURITIS	ARSENICAL NEURITIS
Antecedent history	Conditions affecting thiamine intake or adequacy	Acute upper respiratory disease frequently precedes	Diphtheria 2-3 weeks before	Ingestion of triorthocresyl phosphate	Colic, lassitude, cramps and tremors	Ingestion of arsenic
Onset	Usually insidious	More acute	Gradual	Abrupt	Variable	Slow
Sensory disturbances	Prominent, ascending	In some cases; not severe	Only if motor paralysis is severe	Not marked	Minimal or none	Prominent
Deep reflexes	Slowly disappear	Absent or greatly diminished	Disappear early	Ankle jerks lost, knee jerks remain	Lost	Slowly disappear
Distribution	*Starts distantly in lower extremities*	*Thigh and lower trunk. Proximal parts most affected*	*Related to location of primary lesion*	*Almost invariably of toes and ankles. Fingers often weak*	Extensors of hands and wrists.	All four extremities
Vibratory sensation	Lost	Not impaired	Not affected	May be *diminished*		Lost
Spinal fluid	Normal or with slightly increased protein	Protein usually more than 45 mg. May show lymphocytes		Protein moderately		
Other characteristics	Promptly affected by rest. Cranial nerves intact	Facial nerves bilaterally involved. May be febrile	Paralysis of sphincters is frequent	Paralysis preceded by pain	*Characteristic distribution.* Much atrophy	Trophic changes in skin, hair and nails

mine depletion or the demonstration of response to specific therapy. The edema does not occur as an isolated symptom. The nutritional edema due to protein deficiency occurs in the feet and often in the face. The patients are wasted and asthenic.

A number of special tests have been used as aids in diagnosis. *Aalsmeer's*

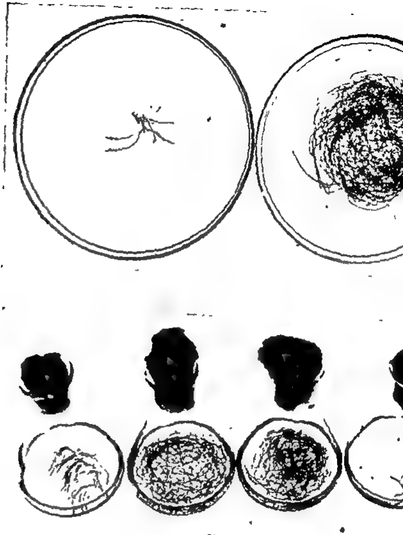

PLATE XIV Vitamins and plant growth. Various vitamins are quit
to plants as to animals. This is well illustrated by these photograp
the effect of thiamin and its intermediates on the growth of excised
cultivated in a solution of mineral salts and brown sugar. The upp
shows the effect of thiamin which has caused an eightyfold increase in
lower photograph is of a similar experiment in which the media was enri
from left to right) with thiazole, thiazole and pyrimidine and with
this case the intermediates are as satisfactory as thiamin itself. V
differ in their requirements and powers of synthesis and this may be u
fying certain vitamins and in assay (Reproduced by permission of D
bins and The Botanical Gazette.)

test, the precipitation of the cardiac symptoms of acute beriberi by the injection of adrenalin (or pitressin—*Wenckebach's test*) may be dangerous but is considered very revealing in mild cases. The failure to excrete water (*Volhard's test*) has been used to demonstrate beriberi. A liter of water is given and the urinary output measured during a period of four hours. Failure to excrete an equivalent amount is presumptive evidence. Since water retention in beriberi is rapidly corrected by treatment this as well as the electrocardiographic changes can be used as criteria of a therapeutic test. Of the chemical tests the measurement of blood pyruvic acid is most readily adapted to clinical use and abnormal levels either at rest or after exertion imply pathological carbohydrate metabolism. The test cannot be considered specific unless other illnesses which also increase the pyruvic acid value can be excluded.

Laboratory Aids in the Diagnosis of Thiamine Deficiency

Thiamine has no properties which lend themselves to delicate testing. The thiochrome reaction is subject to various errors although Westenbrink and Goudsmit reported its successful use in testing urine. The method of Prebluda and McCollum, using as reagent a solution of para-aminoacetanilide and nitrous acid, produces a purple-red compound with thiamine but its application to biological materials is impractical. The amounts of thiamine are, of course, extremely small. Using these methods Schneider and Burger estimate the thiamine content of the urine of normal persons to be between 80 and 100 γ daily and of the blood serum 6.4 γ per 100 ml.

A simpler procedure is Schopfer's test, based on the influence of thiamine on the rate of growth of the mold *Phycomyces blakesleeanus*. This has been used rather frequently in studies of biological specimens. Its limitations are discussed elsewhere in this volume. Rowlands and Wilkinson, using the Phycomyces test determined the blood level of normal individuals to be between 6.5 and 16.5 γ per cent. Lower values were found in cases of neuritis, scurvy and malnutrition (3 to 4 γ per 100 ml.). Other studies of the vitamin excretion have been reported by Harris et al. who used the rat bradycardia method. Good correlation between estimated intake and excretion was secured.

A test which has been used considerably in studying fowl polyneuritis and beriberi is the pyruvic acid test. The bisulphite-binding substances of the blood are determined by a micromethod. This is not specifically due to pyruvate but under experimental conditions is quite dependable. Platt and Lu were able to measure the progress of human cases by means of this test. The objection to it in clinical work appears principally to be its limitation to severe grades of deficiency. Wilkins, Taylor and Weiss tested the blood values of many patients and found it nonspecific for thiamine de-

ficiency. Nevertheless the test serves as a useful measure of the disease and with certain limitations as a diagnostic procedure. The pyruvate level

TABLE XXXII

The Thiamine Content of Human Tissues

(micrograms per gram tissue)

	HEART	SKELETAL MUSCLE	LIVER	KIDNEY	CEREBRUM
Poorly nourished..........	0.6	0.1	0.5	0.5	0.5
Fairly well nourished.......	1.5	0.3	0.9	1.0	1.0
Well nourished..............	2.4	0.4	1.3	1.4	1.1
Thiamine treated...........	3.1	0.7	1.6	1.6	1.2

(Based on results of Ferrebee, Weissman, Parker and Owen.)

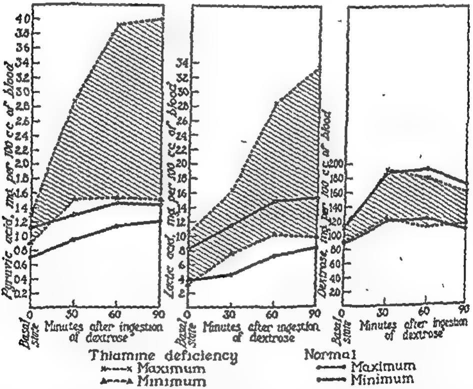

FIG. 37. The range of values of pyruvic acid, lactic acid and dextrose in the blood after the oral administration of 50 gm. dextrose at zero time and again after thirty minutes in thiamine deficient and normal adults. (Courtesy of Dr. Russell M. Wilder.)

in the blood is normally 0.5 mgm. per 100 ml. In mild forms of deficiency the increase may not be considerable but will be excessively increased by muscular exertion and will require an abnormally long time to return to the

resting level. Heavy work causes an even greater increase and may precipitate symptoms of beriberi. In severe, fulminating cases of the disease the resting level is high and, according to Platt and Lu, furnishes a measure of the severity of the illness. We have used the method of Bueding and Wortis with success (Chapter XXVIII).

The blood concentration of pyruvic acid following the ingestion of dextrose was used by Wilder and associates in following the development of beriberi in volunteer subjects (see Fig. 37).

Goodhart and Sinclair found blood cocarboxylase to be a good index of the degree of saturation of the body with both cocarboxylase and thiamine. Comparison was made with biological assays. Cocarboxylase was determined by the method of Ochoa and Peters. The blood cocarboxylase is present only in the blood cells. Thus in conditions such as polycythemia erroneous results will occur. In a more recent report Goodhart states that the blood levels for "normal" boys is 7.0 $\gamma \pm 1.53$ S.D.

TREATMENT

The important element in treatment is, of course, the administration of thiamine. Doses of 10 to 20 mgm. daily appear reasonable. This is much more than the calculated daily requirement but it is known that in experimental thiamine deficiency, also, 3 to 5 times the maintenance dose is necessary to cure. Many cases, especially forms of neuropathy secondary to other diseases will only respond favorably if the dose is still greater and since thiamine is not toxic this is well worth trying.

The response is gratifying and in severe cases dramatic. Hawes states that terminal cases, with pulse rates above 120 per minute undergo an almost immediate response and that changes in blood pressure appear within an hour or two. In such cases, too, large doses are properly given, especially since their effect persists for some days. Cardiac symptoms are said to improve more quickly than the nervous ones.

The other factor in the treatment of severe or moderately severe cases which is of critical importance in the management of cases with cardiac signs is complete bed rest since activity may precipitate sudden attacks and death. Rest alone is enough to induce a considerable degree of recovery in many cases.

And finally diet is important. Jolliffe believes that certain constituents of the B complex are valuable adjuncts. He also gives very practical advice regarding the prescription of a diet. The rule is to eliminate all vitamin-free or vitamin-poor foods. In the severely sick he gives milk, cream, ground liver, puréed legumes, thin, whole grain cereal gruels and fruit juices.

None of the orthodox cardiac drugs are effective in beriberi and even the

most powerful diuretics are valueless. Bleeding is recommended for critical cases with heart failure.

We have heard of unfavorable effects from the administration of thiamine in patients with obscure nervous disorders. In most cases the evidence is indirect. However, Steinberg reports 3 patients in whom herpes zoster followed medication and he believed other patients have shown smooth muscle spasms because of thiamine. His cases received from 1200 to 2000 units, some intravenously, others orally. In one case treatment was resumed several months later and herpes reappeared.

The symptoms of overdosage in small animals are tetanic spasms, slow and irregular breathing followed by respiratory paralysis. No deaths have been reported from thiamine intoxication. Indeed the human tolerates 500 mgm. daily for many weeks without symptoms and Hecht and Weese estimate the ratio of therapeutic to toxic dose in the monkey to be from 1:7,200 to 1:36,000.

Cases of thiamine sensitivity have been described. Several were apparently due to the preservative added. Such reactions can of course be excluded by intradermal testing.

THIAMINE DEFICIENCY IN THE WESTERN WORLD

It is hoped that the preceding review has left the reader convinced that Oriental beriberi was, and is, a dreadfully serious disease for which we have both cure and preventive although tragically not the cure of the conditions which prevent prevention.

It is hoped, too, that there remains little doubt that much the same disease occurs in our own country. If all this is admitted, one is faced with an obvious discrepancy for we do not have the *deaths* from beriberi that occur in the Orient. And if we do not have the deaths, how severe and widespread a deficiency do we have?

Unfortunately the authors are in no position to say. But a critical review of the literature and a continuous experience with the sick poor and a large necropsy service all suggest that while deficiency of thiamine may be widespread it is somewhat less severe than one would suspect from many reports. Our thiamine problem is of a different order than that of the Far East.

Peripheral neuropathy is a relatively unimportant medical problem. Thiamine heart disease seems so unimportant, *relatively*, that standard works on heart diseases dismiss it with a few lines or at most a single page.

Nutritional Polyneuritis

The two forms of thiamine deficiency common in North America are the neuropathy and the cardiac manifestations. Outspoken instances of both

occur sporadically and clinicians with particular interest in nutrition consider that mild forms, not frequently recognized, are very common. Jolliffe, for example, reports the incidence of polyneuritis among alcoholics as being in the neighborhood of 20 per cent. Most of these cases are presumably very mild. Cardiologists disagree concerning the importance of thiamine deficiency as the cause of heart disease.

At a recent meeting of the Association for Research in Nervous and Mental Disease these conflicting points of view were clearly expressed following a clinical differentiation of the various forms of neuritis by Strauss. Strauss' description of nutritional polyneuritis (by which is meant also alcoholic polyneuritis, polyneuritis of pregnancy and of diabetes) could be almost wholely transposed to the preceding description of mild beriberi as it occurs in the Orient excepting for his discussion of etiology:

"In chronic alcoholism appetite and food intake are diminished; gastro-intestinal disturbances which interfere with absorption of food are common; liver disorders may impede intermediary metabolism; finally alcohol per se furnishes a large number of non-vitamin containing calories. In severe vomiting of pregnancy the actual amount of food assimilated is small; gestation with its increased metabolism increases the demand for nutritional factors. In chronic diarrheas whether due to bacteria, parasites, sprue, ulcerative or mucous colitis, absorption of vitamin is impaired. A similar process occurs in gastro-intestinal fistula, pyloric obstruction, cancer of the stomach or bowel. In liver diseases intermediate processes in the handling of food factors may be impeded. In fevers of long duration, hyperthyroidism, and in prolonged strenuous physical activity, demands for accessory food factors are increased. Finally in many of the above states dietary intake is limited qualitatively or quantitatively because of anorexia, economic status or the prescription of special diets. *Thus nutritional polyneuritis develops in the Western World.*" (our italics)

Strauss' description of the clinical features of this form of neuropathy is so complete and clear it is reprinted in full:

"The onset of nutritional polyneuritis may be sudden, but it is generally insidious. Heaviness of the legs and tenderness of the calf muscles when they are squeezed are usually the earliest manifestations. Walking short distances is unimpaired, but when longer walks are attempted weakness may become apparent. Frequently patients will note that they will commence walking with no disability whatever but that after having traversed a variable distance their legs will suddenly collapse under them. At first a distance of a mile or more may be required to bring out this weakness. Later a hundred feet may be sufficient. After some minutes of rest, walking can be resumed. It is improbable that these manifestations are the result of organic degeneration in the nerves. More likely they represent a meta-

bolic disturbance in nerve or muscle due to vitamin deficiency. Burning o the soles of the feet and numbness of the dorsum and lower part of the ankl are next to appear. Weakness of dorsi-flexion of the toes then becomes ob jectively demonstrable. The archilles and patellar reflexes become di minished, then absent. Weakness gradually spreads upward, involving th extensors of the foot, then the muscles of the calf, and, finally, the extensors and flexors of the leg. By the time the thigh muscles become weak, toe and foot drop is usually manifest. With the motor phenomena there is pari passu an ascent of sensory signs. Hypesthesia advances up the leg and thigh in a bandlike wave, with anesthesia following in its wake. Vibratory sense is almost invariably lost and may be the last to reappear when recovery occurs. Not infrequently it remains absent or diminished years after the other manifestations have gone. Atrophy of muscles and skin sets in shortly, the skin becoming smooth and shiny. Not until the disorder has progressed to a moderate degree in the legs do symptoms referable to the upper extremity ordinarily appear. However, the hands and arms may be affected first, especially in individuals who use their hands a great deal more than their legs. As in the feet, the symptoms commence with burning, numbness and weakness of the hands, followed by wrist drop, hypesthesia and anesthesia, together with a loss of tendon reflexes. In an advanced case the patient becomes bedridden, suffers great pain even from the pressure of the bedding, and is prone to develop decubitus ulcers. Sphincter control is usually maintained until very late stages. Cranial nerves other than the tenth are rarely involved. Memory defects are common. The patients with mental symptoms (Korsakow psychosis) are generally cheerful and happy in spite of the extent of their disability and discomfort, although marked mental depression may occur.

"The rapidity with which the polyneuritis of beriberi advances varies markedly in individual cases. There is some evidence that in the total absence of antineuritic factors in the diet about twenty days may be required for the appearance of the first symptoms but this is probably largely determined by the previous diet. Should no treatment be instituted, the late stages may be reached within a matter of weeks. However, most patients suffer from a partial and irregular deficiency of the vitamin. Months may accordingly elapse before marked symptoms appear. With temporary increases in the amount of the vitamin ingested, remissions occur; these are followed by exacerbations as the diet again becomes inadequate. Some individuals may continue for long periods in a relatively stationary condition while partaking of diets containing an amount of the [illegible] covery but enough to prevent [illegible] robably represent acute exacerbations precipitated perhaps by infections or excessive physical activity

in persons who have had unrecognized mild symptoms of the disease for some time."

"Spinal fluid examination is generally normal, although the protein may be slightly elevated. Urine and stool specimens are not remarkable. The blood frequently shows a moderate anemia mildly macrocytic in character."

Strauss agreed with Meiklejohn that thiamine is probably not the only factor involved in nutritional neuropathy. Indeed both authors imply that its role is not even dominant and question whether it should be considered the "anti-neuritis" vitamin. We have already reviewed this problem and believe that the anatomical evidence strongly implies that thiamine is the active agent. Bessey emphasized this in discussing Strauss' report.

The problem of poor recovery despite continued and ample thiamine treatment was discussed by Jolliffe who pointed out that mild cases, patients having only tenderness of the calf muscles, plantar dysesthesia and absence of knee jerks, recover promptly under conditions of complete control of the intake of other B vitamins when thiamine is given.

Cardiac Disturbances

Cardiac disturbances due to thiamine deficiency have been present in this country for many years and have, indeed, been recognized for a considerable period. Weiss and Wilkins, however, should be credited with the most complete studies and for emphasizing the frequency and distribution of this disorder. We have already mentioned that Jolliffe estimates the frequency of cardiac involvement in cases with nutritional neuropathy as about [illegible] per cent. Among 900 case records of patients with some evidence of nutritional disturbance Weiss and Wilkins found 85 they believed suffered from cardiac dysfunction. Within the following two years they observed 35 patients among 5,500 medical admissions. It would seem that the condition is not uncommon and the authors emphasize this by pointing out that in their experience it is a commoner cause of heart disease than either congenital heart disease or subacute bacterial endocarditis.

While the cardiac manifestations do not form a constant syndrome certain symptoms are very common. These are:

1. Dyspnea on exertion with palpitation, tachycardia and embryocardia. The tachycardia may change to bradycardia during treatment. (This is true of oriental beriberi.)

2. Gallop rhythm, prominent cardiac pulsations, pistol shot pulse.

3. Heart of normal size or enlarged. Frequently systolic and diastolic murmurs.

4. The dyspnea may be very severe and can appear unexpectedly with great severity.

5. Signs of pulmonary congestion.

6. Arterial pressure usually normal with a tendency to increased pulse pressure.

7. Veins in neck may be engorged.

8. Skin warm, usually of normal color but may be cyanotic.

9. Edema may occur, either dependent or diffuse and is at times severe.

10. Patients with severe failure are prone to *fever which increases the* symptoms.

Jolliffe has stated the case somewhat *differently. He groups the cases* into one of three categories:

1. *Patients with edema and serous effusions* in the absence of congestive heart failure, enlarged heart or recognized factor producing effusions.

2. Edema and serous effusions with signs of heart enlargement and congestive heart failure, and

3. Sudden circulatory collapse occurring with or without previous signs of circulatory failure.

Both authors agree that the older observations are correct, namely that patients with slight nervous involvement are more subject to cardiac failure because they are more active.

Jolliffe has listed three further characteristics of the cardiac cases:

1. A mild polyneuritis is present.

2. Increased or normal blood *velocity* in the presence of congestive heart failure.

3. Rapid *response to specific therapy with complete* and *permanent* reversibility of the circulatory manifestations.

It must be evident that in all those aspects which have been studied the cardiac as well as the nervous manifestations of thiamine deficiency in this country are the same as those occurring in *classical beriberi. In other* words the only difference in clinical practice seems to be the difference in terminology which hardly seems justified. This, with a history of an unbalanced diet, the results of the therapeutic test, presence of other signs of deficiency, absence of heart disease of another kind and the changes in the EKG and their response to treatment were indeed the reasons that encouraged Weiss and Wilkins to designate their cases as beriberi.

The similarity extends to other features; males are 4 times as commonly affected as females, the response to the adrenalin test and the appearance of the heart, post mortem.

The EKG observations are not constant or specific. They have been reported by various authors as follows:

Aalsmeer and Wenckebach: Tachycardia and shortened conduction time (P-R interval 0.12 sec. or less).

Scott and Herrmann. Negative T1 and T3. Some with right and some with left

ventricular preponderance. Low voltages and slight disturbances in ventricular complexes.

Keefer: Negative T waves, right and left preponderance and low voltages

Weiss and Wilkins: Abnormal T waves, prolonged electrical systole (Q-T) and sinus tachycardia

Feil: Inverted T waves. Prolonged systole.

Dustin, Weyler and Roberts. Increased electrical systole. Rapid rate. Low voltage and flattening of T wave in first three leads.

This summary is from the report of Dustin, Weyler and Roberts who point out that these changes occur in various other conditions. The EKG records of deficient rats were studied by Zoll and Weiss. The changes were similar to those in human cases but the rate was slow.

Weiss and Wilkins have made a further useful contribution to the subject by calling attention to the effects of inanition on the heart. These are bradycardia, decreased arterial pressure, lowered metabolic rate and presumably decreased blood flow. These changes are entirely different from those due to thiamine deficiency. They must be considered in nutritional cases with cardiac dysfunction since both conditions may cause fatal cardiac failure.

Waring described cases of cardiac enlargement with edema which responded to thiamine therapy.

Encephalopathy of Wernicke

A characteristic cerebral lesion was described by Wernicke in 1881 which he called acute superior hemorrhagic polioencephalitis. The lesion consists essentially of symmetric petechiae in the wall of the third ventricle, the grey matter about the aqueduct and on the floor of the fourth ventricle. Wernicke believed this the expression of an infectious disease, a polioencephalitis, but recent work suggests that it is an uncommon form of thiamine deficiency.

Alexander has duplicated the lesion in pigeons by thiamine deficiency and similar changes were earlier observed in rats by Prickett. The lesions in the pigeons were located about the ventricular system and consisted of focal degeneration and varicosities of the vessels with associated areas of parenchymal necrosis and frequently of petechiae. They were most common in the paramedian and paraventricular nuclei of the thalamus and hypothalamus, the mammillary bodies, the periductal region of the midbrain, nuclei triangularis and Bechterew of the vestibular nerve and the dorsal nuclei of the vagus. These are the locations of the lesions in man in whom the histopathology is likewise indistinguishable. The lesion has an especial interest in that vascular effects are predominant. Hemorrhagic lesions have been frequently although erratically observed in various organs in thiamine deficiency. Plate XIII.

normal, fine, silky hair, the skin had a pale pink glistening, new skin appearance. The backs of the hind paws, when affected, presented at first an appearance as of a matting of the silky fur of this part, and then looked dull and thickened. Later this matted layer of fur began to fissure and crack and then gradually desquamate, leaving a denuded pale pink, glistening skin.

The shortest period for producing this dermatitis was approximately seven weeks. In addition to the skin lesions they found a stomatitis comparable to that found in pellagra.

The paw and ear lesions are now known to have been due to pyridoxin deficiency but the generalized, dry, greasy scaliness of the skin with gradual loss of hair was due to riboflavin. Those specimens which we have examined showed little more than atrophy with a few round cells in the superficial corium. Wolbach and Bessey, in a preliminary report, describe the initial changes in the epidermis and its appendages. A moderate keratosis appears, atrophy of the sebaceous glands and loss of hair due to separation of the basal portion of the shaft from its anchoring cells. The outstanding effect was found to be associated with the hair follicles and the absence of regeneration of hairs. The follicle has an atrophic wall and for a time forms imperfect hairs. Evidence of recovery was evident 48 hours after feeding riboflavin.

The most interesting observations of riboflavin deficiency relate to the eye. Bessey and Wolbach describe vascularization of the rat cornea. "It precedes all other demonstrable lesions of the deficiency." Keratitis had been previously observed but attention at that time was directed to changes in the lens of the eye rather than the cornea. Bessey and Wolbach fed their animals a diet composed of 18 per cent casein, 4 per cent Osborn and Mendel salt mixture, 2 per cent cod liver oil, 20 per cent sugar, 48 per cent cornstarch (twice extracted with alcohol) and 8 per cent peanut oil. Thiamine chloride and riboflavin free yeast were given as supplements.

After 3 weeks growth ceased and between the 5th and 7th weeks the palpebral fissure became narrowed, the eyeballs sunken, the lids swollen, the tail dry and scabby. The cornea was dull and the skin manifestations of riboflavin deficiency evident. Preceding these signs of deficiency capillaries commenced to grow into the cornea from the vessels of the limbus and within 3 months these newly formed vessels extended more than one-third across the cornea and some reached its center. The vessels were in the form of an anastomosing plexus which lay immediately beneath the epithelium but which later invaded the deeper structures as well. The lesion was rapidly reversed by riboflavin. Turbidity disappeared within 12 hours, within 2 weeks the vascular plexus could no longer be seen clinically although remnants were detected by histological means for 2 months. Simi-

PLATE XVI Vascularization of the cornea due to riboflavin deficiency. Photograph of a rat eye which was injected with india ink to demonstrate the plexus of newly formed blood vessels. (Reproduced by permission of Dr. L. V. Johnson and the Archives of Ophthalmology See Eckardt, R. E., and Johnson, L. V., Arch. Opthal , 21 315, 1939)

lar changes had been observed in vitamin A deficient rats. The relationship was not clear.

The significance of corneal vascularization and its control by riboflavin were reported independently a month later by Eckardt and Johnson who were studying cataract formation. Only 2 of 12 rats which survived prolonged riboflavin deficiency developed cataract but more than half showed corneal vascularization.

Cataract, reported by Day and associates in almost all riboflavin deficient rats, was rarely encountered in Bessey and Wolbach's experiments. Those instances in which it did occur were believed due to litter susceptibility. Thus in extensive and careful studies two completely divergent results were secured for Day was able not only to produce cataract by riboflavin deficiency but seems to have demonstrated almost as complete control of the process by administering the pure vitamin (Day, Darby and Cosgrove). Other results with similar diets include those of Bourne and Pike who found the incidence of cataract to be 31 per cent, György who found no lens lesions and Richardson and Hogan who "rarely saw cataract." The experience of Eckardt and Johnson has been mentioned. It would seem, therefore, that the consensus is against the rôle of riboflavin in the production of cataract. Galactose rich diets induce cataracts in rats but this does not explain the discrepancy. The galactose cataract is not associated with vascularization of the cornea (Eckardt and Johnson) and is not preventable by riboflavin (Mitchell and Cook).[1] Day suggests that others have not used a completely (flavin) deficient diet.

The syndrome of sudden collapse (vide infra) which is such a striking manifestation of riboflavin deficiency in dogs occurs but rarely in rats. Bessey reports having seen it.

CANINE ARIBOFLAVINOSIS

This deficiency disease was first studied by Sebrell and associates. The symptoms, weakness and ataxia, develop rapidly. The animals soon become unable to walk or stand and lie, mentally alert, with spastic extremities Bradycardia and an exaggerated sinus arrhythmia are notable features. Respiration is slow and deep. Coma then appears and the animals die within 12 hours, frequently much sooner. Riboflavin produces a rapid cure.

This dramatic collapse developed as a late manifestation of riboflavin deficiency (never in less than 102 days)[2] and was preceded by an inconstant dermatitis which commenced as an erythema followed by a dry and scaly

[1] Wintrobe et al. announce the observation of cataracts in swine on riboflavin deficient diets. Corneal vacularization did not occur. (Bull. Johns Hopkins Hosp., 75: 102, 1944).

[2] Axelrod et al. have succeeded in halving this incubation period by improvements in the basal ration.

exfoliation. The dermatitis was most common over the chest, abdomen and inner surfaces of thighs and axillae. In male dogs it characteristically involved the scrotum. Anemia was often present but did not respond to riboflavin feeding.

Anatomic study showed fatty degeneration of the liver and, to a lesser degree, the kidney (Henle's loop). Small patches of hepatic necrosis have been found. The bone marrow was atrophic and fatty. Nodular hemorrhagic lesions occurred in the lungs. Extensive and moderately severe degeneration of nerve cells was found in brain and cord as well as myelin degeneration of fibers in the pyramidal tract, median longitudinal bundles and brachium pontis. Myelin degeneration was also seen in the glossopharyngeal and accessorius nerves and in parts of the fasciculus cuneatus.

Similar behavior was noted in B_2 deficiency experiments by Zimmerman, Cowgill and Fox. The etiologic importance of riboflavin in the production of this syndrome was reinvestigated by Street and Cowgill. By using improved diets, deficient only in flavin, they produced the same clinical responses. After 1 to 9 weeks of steady loss of weight and diminished food consumption (which differed from that of thiamine deficiency in its erratic, fluctuating character and the persistence of some eating until the onset of the collapse phenomenon) their animals became apathetic, reluctant to walk, soon staggered and then became semicomatose. Vomiting almost always occurred, some had convulsions and most diarrhea. The diarrhea, however, was a symptom of the collapse stage and was not present during the prodromal period. Complete recovery was effected in 5 of 7 animals within 2 to 3 days by riboflavin treatments. Street and Cowgill demonstrated inversion of the T wave (confirming Sebrell and Onstott), found a fatty liver in one animal but did not observe bradycardia. A pronounced fall in body temperature and respiratory rate occurred during the attacks, the heart rate remaining normal or increasing.

Presumably therefore bradycardia and anemia are due to other than riboflavin deficiency. The syndrome in other respects must have been identical in both laboratories and constitutes a striking clinical manifestation of vitamin deficiency. Whether the dermatitis reported by Sebrell and Onstott must also be accounted for by another deficiency is unknown. As regards the anemia it should be noted that György, Robscheit-Robbins and Whipple found that riboflavin increases hemoglobin production in dogs made anemic by repeated bleedings. The response is of the order of $\frac{1}{4}$ that produced by 300 grams of pig liver.

AVIAN ARIBOFLAVINOSIS

The importance of the growth factor in the B complex for the chick was demonstrated many years ago and has become recognized as extremely important by the poultry industry. In young chicks the most striking mani-

n of chronic riboflavin deficiency is "curled toe paralysis." Pro- partial deficiency is necessary for its production. Complete tion induces an acute condition marked by paralysis and dystonia esembles the disease in dogs. The "curled toe paralysis" com- as gradually increasing flexion of the toes continuing to a stage of s. The disorder is usually bilateral.

esions are confined to the nervous system (Phillips and Engel). er cent of their animals the sciatic nerve was degenerate. Patchy degeneration were also found in the spinal cord involving all por- d tracts. In the legs the nerve end-plates in the muscles are said to en and an occasional muscle fiber is also degenerate but the lesion is se purely nervous.

eggs of hens partially depleted of riboflavin do not hatch (Engel, and Halpin) although the animals may be free of lesions. The de- y be restored by injecting riboflavin into the egg on the first day of ion. The measurements reported by these authors indicate that umin must contain riboflavin in a concentration of from 2 to 3 γ per insure normal development of the embryo.

ovsky and Jukes, in extended studies of riboflavin deficiency, lend to the view previously mentioned that dermatitis is a manifestation lavin deficiency. They failed to produce dermatitis in chicks but he rat (in which the lesion was distinguished from "rat acrodynia") the turkey. In the latter animal the vent becomes encrusted, in- and excoriated. In other respects the disease is similar to that in

HUMAN ARIBOFLAVINOSIS

35 Landor and Pallister reported a disease common among prisoners apore and Johore. The signs were an eczematous scrotal dermatitis, s of the tip and margins of the tongue and stomatitis limited to the of the mouth. Later on most cases developed paraesthesias, weak- d stiffness of the legs. The mouth lesions developed at the muco- ous junction where the skin became white, sodden and heaped up. fissures appeared. The disease was believed due to vitamin B_2 ncy because pellagra was not present in typical form and liver, a and brewer's yeast were curative.

938 Sebrell and Butler observed a similar lesion in the mouths of fed a diet of cornmeal, cowpeas, lard, casein, flour, white bread, n carbonate, tomato juice and cod liver oil. Ascorbic acid and thi- supplements were given. Within 3 to 4 months 10 of 18 women so veloped pallor of the mucosa in the angle of their mouths. The areas e macerated and in a few days transverse, superficial fissures ap-

peared. The lesions remained moist and were covered by a honey-colored crust which could be removed without causing bleeding. Some of the fissures were ½ inch long. The condition was obviously identical, clinically, with *perleche*, a disease previously noted especially among children.

At the same time these patients developed increased redness of the lips (believed due to denudation of the epithelium) and a fine, scaly, slightly greasy desquamation appeared in the nasolabial folds, on the nasal ali and in the vestibule of the nose and on the ears. The lesions did not respond to nicotinic acid but responded promptly to riboflavin.

Subsequently spontaneous cases of the disease were described by Oden, Oden and Sebrell as being quite common in rural Georgia. Three such cases were treated with 5 mgm. synthetic riboflavin. All recovered within a few days. The authors conclude that ariboflavinosis (the name suggested by Sebrell and Butler) is "a common dietary-deficiency disease in the southern states."

Subsequent reports by Spies, Bean and Ashe, Sydenstricker, Geeslin, Templeton and Weaver and Jolliffe, Fein and Rosenblum have confirmed these observations. Smith and Martin, however, treated 4 cases with synthetic vitamin B_6, given intravenously in 50 mgm. amounts. Their first patient responded within 5 hours. After 24 hours improvement was demonstrable photographically. Treatment was withheld and the lesion recurred after 2 days. Smith and Martin state that experimental B_6 deficiency is more similar to cheilosis than any lesions seen in experimental riboflavin deficiency. They also refer to a report by Aykroyd and Krishnam in which cheilosis was said to respond to treatment with a yeast preparation free of flavin. Smith and Martin suggest that both factors may be necessary for the maintenance of the epithelium of the muco-cutaneous junction or that the two are dependent.

The clinical manifestations of riboflavin deficiency also seem to include other characteristic lesions of the face and tongue. Nasolabial seborrhea, often involving the eyelids and ears is common. A seborrhoic and follicular keratosis of the forehead, malar eminences and chin is described by Sydenstricker, Sebrell, Cleckley and Kruse. Fine filiform comedones over the cheeks and chin resemble "urea frost." These authors confirm an observation of Jolliffe that riboflavin deficiency produces a characteristic glossitis. The features are a clean tongue, of a purple red or magenta color and frequently with fissures. The papillae are large and flattened or mushroom shaped. It was formerly thought to be a sign of relapse in pellagra since it occurs in pellagrins after treatment, either because the deficiency of riboflavin first becomes evident after the cure of pellagra or because the lesion is masked by pellagra glossitis. At times a dry, scaly dermatitis of the hands disappears during treatment with riboflavin. This is an uncommon sign.

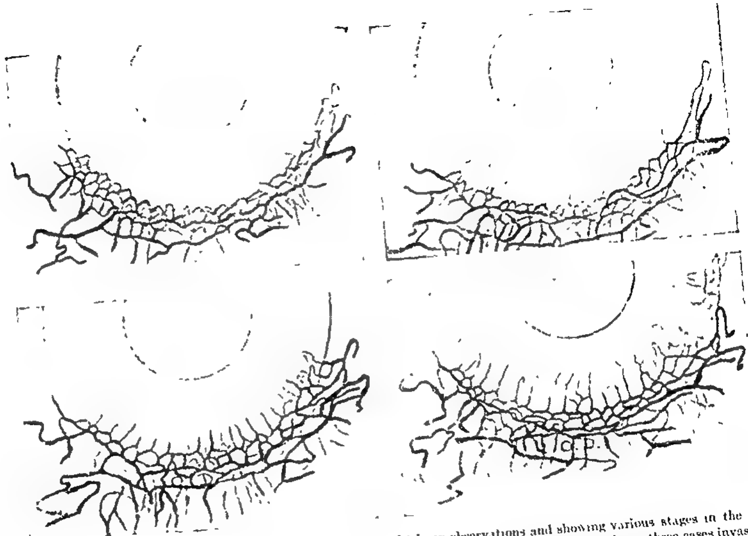

PLATE XVII Human ariboflavinosis Drawings based on slit-lamp observations and showing various stages in the development of the corneal lesion In the upper left the limbic vessels are congested In the remaining three cases invasion of the sub-epithelial region of the cornea by newly formed capillaries is seen (Reproduced through courtesy of Dr V. P Sydenstricker and the J. A. M A.)

PLATE XVIII. Ariboflavinosis Cheilosis, naso-labial lesion and blepharos|
(Reproduced through courtesy of Dr. V. P. Sydenstricker and the J. A M. A

Recently it has become evident that the corneal lesions described by Bessey and Wolbach in the rat have their exact counterpart in man. Ocular symptoms and signs had been encountered in various studies of pellagra. They were ascribed to other deficiencies than nicotinic acid; vitamin A and riboflavin. Pock-Steen found the same manifestations in a large group of sprue cases. Of 109 such patients 78 responded to riboflavin. These and other observations of mixed deficiencies, usually associated with pellagra, have been made the subject of special study by Sydenstricker, Sebrell, Cleckley and Kruse. Among 47 cases of ariboflavinosis the symptoms shown in table 24 were encountered. It is evident from this table that among these particular patients ocular signs and symptoms were more common than the other manifestations of deficiency.

TABLE XXXIII

Frequency of Certain Symptoms in Forty-seven Cases of Ariboflavinosis

	Number of patients
Photophobia	43
Burning of eyes	40
Dimness of vision	29
Burning lips and tongue	27
Seborrhea	25
Cheilosis	35
Glossitis	32
Conjunctivitis	37
Circumcorneal injection	45
Corneal vascularization	37
Corneal opacities	[illegible]
Pigmentation of the iris	19
Iritis	4
Cataract	6

After Sydenstricker, Sebrell, Cleckley and Kruse, J. A. M. A., 114:2437, 1940.

Circumcorneal congestion had, indeed, been noted by many observers in riboflavin deficiency. Inspection however fails to reveal the characteristic feature of the lesion which is only evident, during life, by slit lamp. By this means the circumcorneal injection may be seen to consist of an extreme congestion of the limbic plexus of vessels with proliferation of new vessels. This stage is followed, if the disease progresses, by radial invasion of the cornea by newly formed vessels just as it occurs in the rat. In many cases superficial nebulae are associated. Interstitial or posterior nebulae are much less common. In advanced cases the penetrating vessels invade the deeper layers of the cornea, but never to the degree that they do in the superficial region.

Prompt response, both of the symptoms and also the signs, followed doses

of from 5 to 15 mgm. of riboflavin. The vascular plexus commenced to shrink within 48 hours. In 1 case the vessels emptied within 24 hours. Extensive lesions required 5 to 18 days to regress.

The order in which these various lesions appear is evident from cases allowed to relapse. The first sign to reappear was conjunctival injection, followed by photophobia and impairment of visual acuity. Corneal opacities were encountered 7 to 10 days after re-instituting a deficient diet. Cheilosis and glossitis seldom recurred before the end of the second week.

In another report on the ocular signs of deficiency Kruse, Sydenstricker, Sebrell and Cleckley state that 2 cases of syphilitic keratitis which were treated with riboflavin also benefitted greatly and the authors raise the question whether syphilis directly causes keratitis or whether it disturbs the riboflavin metabolism.

A form of corneal dystrophy, *essential corneal epithelial dystrophy*, characterized by dots and small streaks of grey in the corneal epithelium which tend to extend transversely across the cornea in a double line just below the pupil and which readily stain with fluorescein is reported to be common in Trinidad and to respond to riboflavin treatment (Métivier). Photophobia and lacrimation are associated symptoms. Hou found ocular lesions of ariboflavinosis relatively common among patients attending an eye clinic in China. Phlyctenular conjunctivitis was frequently associated.

The suggestion that sudden death in man may be due to the mechanism associated with collapse in dogs has been discussed elsewhere (page 253).

CHAPTER XIX

NIACIN DEFICIENCY

Niacin deficiency has great killing power. It is by far the most destructive of the avitaminoses in North America and is a more dangerous disease in many respects than beriberi. Yet the human requirements of niacin, or nicotinic acid, are relatively small. The National Research Council recommends 18 mgm. for adults, 23 mgm. under conditions of great activity. Howe reports that the consumption in a number of Army camps has been maintained at approximately 27 mgm. by diet alone. Cheldelin and Williams calculated that the per capita consumption in the United States during the period 1934–1937 was 11 mgm. and that the enrichment of white flour has increased the average consumption to 17 mgm.

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The estimates of human requirements are based largely on studies of the dog's requirements. The nicotinic acid content of the blood and urine in pellagra is not different from that in health and tolerance curves have failed to add to our knowledge of human requirements. In experimental animals the concentration in the liver is a useful guide. As estimated by the National Research Council's Committee on Food and Nutrition the requirements by age and sex are 10 times the requirements of thiamine.

PELLAGRA

The morbid consequence of prolonged nicotinic acid (now officially named *niacin*) deficiency is pellagra, a disease characterized clinically by dermatitis, stomatitis, gastro-intestinal and nervous symptoms and anatomically by degenerative lesions of these structures. The disease occurs spontaneously in man and the dog and probably in other species as well.

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Pellagra was first studied by Don Caspar Casal in 1725 in the province of Asturia, Spain, where an apparently new disease was prevalent among the peasants. It was then known as *mal de la rosa*. Casal first thought it was atypical leprosy, "scorbutic leprosy." His studies were not published until 1762. Nine years later the disease became prevalent in Milan and was investigated by Frapoli who first called it pellagra, a corruption of the Italian description *pelle agra* or rough skin. For a generation pellagra remained the most serious disease in southern Italy and outstanding clinical and ex-

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It has been suggested that the coproporphyrin concentration, which roughly parallels the evolution of the clinical symptoms, may create photosensitivity but equal concentrations occur in other diseases in which dermatitis does not appear. The relationship between exposure to strong sunlight and pellagra is not limited to the skin lesions. Patients in remission will suffer exacerbation of mouth and gastro-intestinal symptoms as well as dermatitis if exposed to intense solar irradiation. In some cases dermatitis has been observed to appear after exposure to a hot stove (Ruffin and Smith).

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pellagra seems due to economic conditions which are associated with poor hygiene and which are commonly perpetuated from one generation to another.

Etiologic factors which now are of only historical interest are virus infection (Tucker), intestinal toxins elaborated by certain organisms common in the stools of pellagrins (Jobling and Arnold) and the influence of maize. The close association of corn in the diet and pellagra has never been completely explained. Early Italian physicians were greatly impressed by it and Funk compared pellagra and beriberi on the basis that in the former the diets were predominantly of corn and in the latter of rice. Handler reports that blacktongue develops more rapidly on a corn meal diet than its equivalent in synthetic constituents. Three of 11 dogs remained well on the latter ration. This should be understood to be but the most recent reappearance of the maize theory of pellagra. What the actual effect of corn is remains unknown but presumably it does exert an influence on the evolution of the deficiency state.

The occasional case of pellagra in which the background does not indicate a restricted diet has often served to arouse doubts of the dietary nature of the disease. Orton and Bender's case in which lesions in the lateral horns were found is of this kind. Orton and Bender suggested that the lesions were the predisposing factor. Goldberger was well aware of these isolated exceptions. In 1916 he wrote: "We have investigated a number of such cases and have found that although there may indeed have been a rich, varied diet on the family table, the patient, by reason of some personal idiosyncrasy, did not actually eat it. In other words, it is assumed in such instances that the diet of the family (or the institution) is the diet of the individual, an error that is responsible for much of the misconception and confusion in current discussions of the role of diet in the causation of pellagra."

Smith, Persons and Harvey subsequently reopened the possible effect of fuso-spirochetal organisms in producing the mouth lesions of pellagra. The same flora was found in black tongue disease and dogs on the Chittenden, Underhill and Mendel diet (vide infra). This is a perennial theory in oral pathology which does not agree with the facts of the case as we trust the further discussion of the subject will show. The studies of Topping and Fraser (page 257) are in agreement with the almost universally held opinion that these organisms are not important in the production of oral lesions but simply complications of lesions produced by other means.

None of these theories explain the sudden spontaneous remissions of [illegible] Thus [illegible] nd then [illegible] abruptly recover without change in diet. Under experimental conditions the signs invariably reappear if the deficient diet be maintained.

PLATE XIX Pellagra The early lesions in the skin Photographs of a biopsy sample showing rarefaction of the corium with fragmentation of some of the superficial collagen

THE MORBID ANATOMY OF HUMAN PELLAGRA

The appearance of persons who have died of pellagra is characteristic only to the degree that appearance of lesions of skin and mouth are characteristic. Emaciation occurs in late cases but the bodies may be in good fat. The viscera, other than the gastro-intestinal tract, give no clue to the nature of the disease present.

Three systems are structurally altered in pellagra: the integument, the gastro-intestinal tract, and the nervous system. Excepting for the early stages of the disease and the skin and colon lesions in this period, the lesions are usually nondescript and throw little light on the pathogenesis. The early skin lesions are therefore of particular interest and value in the anatomical diagnosis of pellagra.

They were originally described by Denton to whom the following account is mainly due. In addition to Denton's own report of his observations, which were made on carefully selected material studied in Panama and in which the autopsies were performed very soon after death, we have reviewed his histologic material and compared it with many cases of our own. Moreover, it has been our practice for some years to examine biopsy material from the margins of pellagrous skin lesions and several cases of this sort have also been reviewed.

Skin

The first change in the appearance of the skin seems to precede the erythema and, in our experience, has been found immediately adjoining the erythematous patch. The lesion commences in the corium and consists of edema of the papillae, dilatation of the papillary blood vessels, and deterioration of the superficial—fine collagen—layer of the corium. Slight edema in the deeper portions of the epidermis occurs. Denton saw increased rate of multiplication of the cells of the basal layer as well, but this has not been conspicuous in our own cases.

The rarefaction of the corium may be quite pronounced but it is probably evanescent. The sequences have never been accurately worked out because of the paucity of graded material. In well developed lesions the capillary endothelium is swollen and the finer collagen fragmented, often lying in brilliant eosinophilic dots. A few eosinophiles lie in the edematous zone.

It is perhaps significant that the lesion is so sharply limited to the narrow junction zone between the corium and epidermis since this appears to be a region of highly specialized tissues, rich in nerve fibrils and cells related phylogenetically to the nervous tissue—chromatophores. It is possible that damage occurs during the acute stage of the lesion which qualifies the character of the epidermis thereafter.

Rousentoul has described changes in the nerve fibers of the skin in early

pellagra. The first change was an intense argentophilia and occasionally spindle shaped swelling of the fibers. The later changes became more pronounced in older lesions in which fragmentation of the axis cylinder was also seen.

Following the early stages of the lesions vesicular formations in the epidermis may occur, and if they do they usually become infected and the epidermis sloughs off, at times in shreds. This in turn is followed by the late stages in which the superficial corium is either atrophic and inconspicuous or may have a thickened horny layer with loosely adherent lamellae. The rete cones are irregular and often elongated and collections of round cells are common in the papillae about the vessels. The character of the pigmentation is disputed. Formerly it was believed that the chromatophores are increased in numbers and that increased melanin is present in the rete malpighii and basal layer of the epidermis. Herzenberg states that in her experience this has not been so but that many granules of an iron pigment have been present in the epidermis, chiefly in the stratum granulosum.

The oldest lesions are characterized by atrophy of the rete malpighii, the cells of which are also reduced in number and limited in size. The epidermis is thin. The skin appendages are not affected in pellagra. Moore, Spies and Cooper were unable to distinguish the lesions of pellagra from dermatitis due to various other diseases.

The oral surfaces are affected like the skin in the early stages of pellagra and show the same erythema. The buccal mucosa sometimes shreds off in sheets or patches of white necrotic epithelium. The tongue may become atrophic and smooth.

Gastro-intestinal Tract

A macroscopic lesion of the stomach occurred but once in Denton's cases. In that instance a large false membrane was present near the cardia. In two cases enteritis was found in the ileum and the small bowel was uniformly dark red in color.

The colon, however, was consistently altered, its walls thickened, red in color, covered with patches of pseudo-membrane and stippled with small gray bodies. Histologic examination showed the latter to be cysts formed of distended crypts of Lieberkuhn, their lining cells flattened and compressed; their contents retained secretions. The intestinal glands were reduced in number.

According to Herzenberg the cystic lesions are characteristic of pellagra and practically pathognomic. They occur in only one other disease—sprue—and there infrequently. The demonstration of such changes in the colon is therefore of considerable significance to the pathologist since they afford a second basis for an anatomic diagnosis of pellagra. In earlier pathological

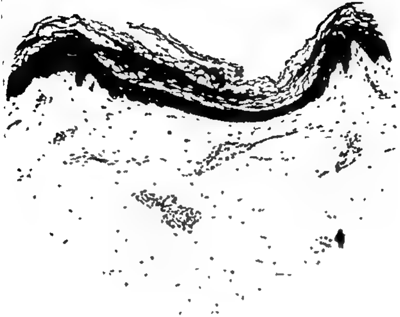

Fig. 1

Fig 2

PLATE XX Fig 1 An old, pigmented and atrophic skin lesion in pellagra. Fig. 2 An acute buccal ulcer in pellagra Membrane lies to the right The epithelium persists in the left From a specimen of Dr James Denton

Fig 1

Fig 2

PLATE XXI Two lesions in the colon from human cases of pellagra Figure 1 is a photograph of a specimen from the collection of Dr James Denton and illustrates the cystic glands which are characteristically found in pellagra, sprue and possibly other related deficiency diseases The lesion was formerly known as colitis cystica superficialis and was associated with malnutrition Figure 2 shows the margin of a chronic ulcer of the colon in a case of pellagra of long duration The ulcers are superficial and shallow.

ntings the lesions were identified as colitis cystica superficialis and even en they were recognized as being regularly associated with prolonged dietry deficiency. Nothing is known of the mechanism involved in the production of these lesions. They have recently been observed by Lucksch in ses of pellagra, and it is interesting to note that similar changes were deribed in the fundus of the stomach in Addisonian anemia by Meulenacht.

In Denton's cases the surface of the colon was intact save for patches of perficial necrosis, but the mucosa was infiltrated with plasma cells, lymnocytes, eosinophiles, and endothelial cells. Areas of acute inflammation d hyperemia, and edema were common. Some deterioration of the intinal ganglia was noted. The late stages of the lesions of the colon are aracterized by atrophy of the mucosa and the appearance of superficial, times cicatrized, ulcers.

Nervous Lesions

Denton minimized the nervous lesions, considering them a by-product of disease. This was a natural deduction to draw from his material which s largely composed of acute cases.

The nervous lesions appear relatively late. There is adequate evidence show that nervous lesions are present in pellagra and indeed may indicate isturbance predominantly of the nervous tissues of which skin and intesal lesions are but two expressions. Unfortunately the lesions of the nerv- system are not specific, consisting of areas of axonal degeneration of the amidal cells of the cortex and degenerative lesions in the spinal cord.

he only difference between the nervous lesions of beriberi and pellagra e distribution. Central lesions are common in the latter and rare in the ner, where peripheral lesions are more pronounced.

he cerebral effects are irregularly distributed, the frontal lobe being said e more constantly involved than other parts. The large pyramidal , in scattered foci, show chromatolysis with displacement of the nucleus fat accumulations. Hyperplasia of the macroglia may occur.

he spinal cord lesions consist of myelin degeneration of both endogenous exogenous fibers and are most marked in the posterior columns, espe- y the columns of Goll. The peripheral portions of these structures are, ever, frequently spared. Orton and Bender emphasized the older char- of the lesions of the lateral horns in the case they studied. Loss of e cells and extensive fibrous glial replacement were present. Tucker found these areas extensively involved and, since they represent the l sympathetic ganglia, the lesions may be related to some of the symp- of pellagra.

e characteristic of the lesions of the spinal cord is their irregular dis-

tribution. Various tracts may be affected and, since in late cases considerable gliosis may be present, the lesions resemble those of subacute combined sclerosis. It is probable that these two conditions, pellagra and subacute combined sclerosis are related etiologically as well as anatomically. The relationship of the latter to pernicious anemia and its occurrence in cases of pellagra in which achlorhydria occurs, both suggest such a connection.

Lesions in the roots of the cord are infrequent and then usually confined to the posterior roots. In a few cases demyelinating lesions of the peripheral nerves are found. *The weight of experimental evidence indicates that the nervous lesions are not due to nicotinic acid deficiency but to associated dietary defects or other causes.*

None of the other organs are significantly altered excepting only the liver. It is almost regularly affected, usually of a dirty yellow color and often mottled. It is not enlarged. Microscopic examination reveals a slight interlobular fibrosis, accumulations of round cells in the periportal spaces and fatty degeneration and cloudy swelling of the hepatic cells. The heart is not enlarged or otherwise diseased.

EXPERIMENTAL PELLAGRA

Goldberger, Wheeler, Lillie and Rogers commenced to use the dog for the study of experimental pellagra because a disease of dogs known as black tongue occurs spontaneously and is similar to human pellagra. Dermatitis has been produced in rats and chicks by elimination of certain vitamin B fractions, but it is improbable that these dermatoses are true analogues of human pellagra.

Shortly after Denton's report on the early lesions in human pellagra, he was intrusted with the examination of the dogs used at the United States Hygienic Laboratories. He examined sixteen animals, at least half of which had early, acute "black tongue," one of which was a normal control and others of which had had recurrent attacks of the disease.

Denton found in these dogs the same distinctive lesions that he had observed in Panama in human cases of pellagra. Lillie has since extended these observations by describing the changes in the viscera not so characteristically affected, and Crane-Lillie and Rhoads, and Zimmerman and Burack have reported anatomical studies of the nervous lesions of "black tongue." Rhoads and Miller have reported special studies of the effect of the black tongue producing diet on the morphology of the blood and bone marrow. The following description is drawn chiefly from these sources.

The Lesions of Black Tongue Disease

Denton found that the sequences in the epithelium could be most advantageously followed in the mouth. There early lesions may occur before mal

nutrition is evident in the animal's general condition. On the floor of the mouth or checks, or along the inner side of the upper lips, areas of redness appear, at times with slight elevation of the surface. These areas are dark red or greenish gray due to superficial necrosis and membrane formation. When the disease is well advanced the entire lining of the mouth and pharynx becomes deep red, swollen and stippled with patches of false membrane.

The early lesions in the mouth are fleeting; in a few chronic cases they become more persistent. The lesions are commonest first near the canine teeth, and then may extend as a brilliant red streak completely encircling the alveolar arches.

Once necrosis starts, a fetid odor and drooling appear with stringy, egg white-like secretion hanging from the corners of the mouth.

The histologic changes are similar to those seen in human skin lesions, commencing as a thin zone of rarefaction just beneath the epithelium with increase in the thickness of the zone due to the presence of an albumin-poor transudate. The collagen fibrils which form the matrix of this part of the skin become slender and fragmented. The vessels are distended, their endothelium thin. Subsequent changes are secondary to the lesions of the corium and consist of epithelial and cicatrical changes in the corium associated with infiltration by small lymphocytes. Loss of epithelium may result in infection.

Lillie examined the nerves of the lip and found myelin sheath degeneration in from one-sixth to one-third of the fibers in all but two of twenty-six animals.

The acute lesions are difficult to follow on the superior surface of the tongue and in the pharynx, due to structural differences in these tissues. Presumably they are analogous. The esophageal lesions were similar to the buccal ones.

The gastro-intestinal tract shows little evidence of disease on inspection although in some cases it is reddened. More may be learned from histologic examination. The stroma of the villi becomes rarefied and distended with a thin poorly stained fluid. In the colon the lesions are more pronounced, the villi there being greatly distorted and cyst formation in the crypts of Lieberkuhn commonly found.

Skin lesions in black tongue seem limited to the skin of the scrotum. Clinically these lesions are similar to the dermal manifestations of pellagra. Denton found microscopic evidence of the disease also.

The nervous system was not comprehensively studied in Denton's work and no significant lesions were found in any other organs. Lillie found a slight degree of degeneration in the myocardium and in one animal fatty degeneration. The great vessels were normal. Many of his animals had inflammatory lesions in the lungs, a change also present in some of Denton's

cases. Lymphoid atrophy is often seen in the spleen. Degenerative changes, of slight degree and extent, may occur in the kidneys.

Dogs which suffer from a chronic, recurrent form of black tongue, develop a striking atrophy of their tongues and sometimes a moderately severe macrocytic anemia which, when it does occur, seems related to the process of black tongue (Rhoads and Miller). The same authors, with a slightly modified diet, produced an acute pellagra-like syndrome which at times was associated with more severely ulcerative mouth lesions and extreme leucopenia and a maturation defect of the bone marrow.

The nervous lesions in black tongue are degenerative, as is true of all the lesions due to deficiency of fractions of vitamin B which have been studied. Myelin degeneration and axonal reaction are both found. Both the central and peripheral nerves are affected. The fasciculus gracilis is the most commonly and extensively affected tract (Zimmerman and Burack), but the posterior columns are affected in many cases. These authors considered the lesions to be identical with those in human pellagra. While epithelial lesions may develop in one or two months, the nervous deterioration is relatively late, apparently requiring nearly twice as long.

A frequently recurring observation in the writings of students of experimental pellagra is the variability in the response of different animals. This is much less common in the production of acute black tongue, however, than of the other resulting manifestations of the diets used. Denton's material seems to have run remarkably uniform, especially the group of dogs fed the basal diet alone.

The Chittenden, Underhill, Mendel, "Black Tongue"

A discussion of the pellagra syndrome in dogs would not be complete without reference to a similar stomatitis produced by these workers. The disease was characterized by abrupt onset after a feeding period of from one to eight months. The dogs developed pustules on the inner surface of the cheeks, lips, and on the edges of the tongue after initial symptoms of apathy and refusal to eat. The mouth became so foul and so covered with pustules it resembled rotten meat, and pustules sometimes appeared over thorax and upper abdomen as well.

Anatomical studies made by Lambert showed the buccal mucosa to be red, covered with a fibrino-purulent exudate and the redness to extend over pharynx and upper esophagus. The intestinal tract was injected, particularly the large bowel

Microscopic study showed the lesions to be very superficial with slight cellular reaction. The first response of the tissues appeared to be hyalin degeneration and swelling of the epithelial connective tissue followed by a

sloughing of the epithelium. Lambert felt the intestinal glands might be atrophic.

Underhill and Mendel illustrated their report with pictures of the gross appearance of the mouth lesions which closely resemble those seen in black tongue. However, no photomicrographs are available by which the minute structure of the epithelium may be compared with pellagra and, as Goldberger has pointed out, scrotal lesions did not occur in Underhill and Mendel's animals as they commonly do in black tongue. Moreover, the dermatitis on the chest is foreign to the latter.

Since carotene completely controlled and prevented the Underhill-Mendel lesions, and since proof of the identity of the two conditions is lacking, it seems probable that the two diseases are essentially different though grossly similar. The syndrome would seem to be very similar to the combined deficiency of vitamins A and B which has been described in man by Wright who has designated the syndrome "polyavitaminosis A and B." The histologic criteria should be applied to all future forms of experimental pellagra for much confusion now exists which microscopic study could presumably clarify.

Pellagra in Pigs

The Goldberger-Wheeler diet is capable of producing a pellagra-like condition in pigs. Young animals develop loss of appetite, retarded growth and slowly developing diarrhea. Their skin becomes scurfy, with scabby patches especially common on the backs of the ears. Death usually occurs within one month after the appearance of the diarrhea and post-mortem examination shows a pronounced inflammation of the caecum and other parts of the colon with a plastic exudate. Glossitis and stomatitis do not occur. The condition has been studied by Birch, Chick and Martin. These authors report that, while the colitis is intense enough to suggest infectious disease, yeast is curative.

Pig pellagra has been frequently used in the study of the disease. It assumes added interest in view of the experience of Madison, Miller and Keith who investigated an unthrifty herd of pigs in Pennsylvania. Of 76 animals 40 had died The survivors were listless, ate poorly, suffered from diarrhea, and had a heavy, scurfy dermatitis of the body and ears. The similarity of the condition to the experimental disease in pigs led to a trial of nicotinic acid in daily doses of 50 mgm. Prompt recovery occurred. Presumably pellagra occurs naturally in the pig as well as the dog.

Harris has fed monkeys a similar diet. Within a few days to a week the animals became patently ill, appetite failed and a profuse diarrhea soon followed. Some dryness and roughening of the skin was noted. 5 to 25 mgm. of nicotinic acid daily was curative. Clark has described a small experi-

gurgitation of whatever food is eaten are later digestive symptoms. Many cases (40 per cent) develop achlorhydria.

In persons with such symptoms the presence of the skin lesions is ample evidence of the presence of pellagra. The diagnosis can never be made with certainty in the absence of the dermatitis. The most important features of the skin manifestations are the sharp margins, the bilateral symmetry, the manner of their evolution, and the pigmentations and keratosis.

The lesions commence as dark red areas which become confluent and gradually browner in color. Scaliness develops during the erythematous stage with thickening of the skin and sometimes bullae and vesicles. Burning and

TABLE XXXIV

Frequency of Certain Cardinal Symptoms of Pellagra

SYMPTOM	FREQUENCY
	per cent
Loss of weight and strength	100
Dermatitis	96
Glossitis	82
Tachycardia	76
[illegible]	66
[illegible]	62
[illegible]	62
Anemia	[illegible]
Diarrhea	58
Vomiting	56
Mental symptoms	54
Achylia gastrica	40
Other diseases	30

Based on 50 cases of severe endemic pellagra. Spies, T. D., Chinn, A. B., and McLester, J. B., J. A. M. A., 108:853, 1937.

itching may be associated with the early lesions. The latter stages show a tendency to atrophy with a senile, parchment texture of the skin.

The distribution of the lesions is characteristic. The commonest sites are the dorsal surfaces of the hands and lower forearms, and the neck. The former has a gauntlet pattern. On the neck a necklace formation is frequent (Casel's necklace). The dorsum of the feet and lower legs are sometimes affected in persons habitually barefoot. There may be patches over the sternum and on the labia or elsewhere in which cases the lesions are usually associated with mechanical irritation.

Bedridden patients may develop hyperkeratotic, pigmented lesions over the bony prominences which, in their lack of vesiculation and slower evolution, differ from the characteristic dermatitis. Such lesions are not subject

PLATE XXII Pellagra Upper photographs show dermatitis of face and hands. The lower photograph shows Casel's necklace

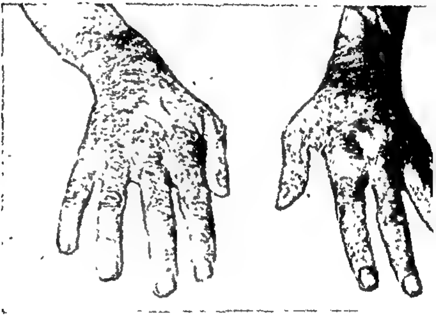

PLATE XXIII. Pellagra   Dermatitis which developed on the hands

to exacerbation when the constitutional symptoms are aggravated. Their relation to niacin deficiency is obscure.

The scrotal lesions of pellagra require special mention because they indicate a current source of uncertainty in our knowledge of the disease and also because they have historical interest. Goldberger and Wheeler's experimental human pellagra, produced at the Rankin Farm of the Mississippi State Penitentiary, was noteworthy for the frequency of this lesion. Of the 11 volunteers fed the experimental diet, 6 developed skin lesions. All of them had lesions of the scrotum and Goldberger and Wheeler were led to believe that scrotal lesions were the earliest dermal manifestations of the disease. At that time only 2 reports of such lesions existed, one by Stannus, writing from Nyasaland, Africa. Stannus found, among 100 cases of pellagra with eruption, 19 with scrotal lesions. Of these 4 had lesions only of the scrotum. Goldberger was able to find scrotal lesions with similar frequency in South Carolina and a special investigation supported his opinion that the lesions were frequently the first dermal sign of pellagra. However the great frequency of the lesions among the 11 volunteers remained a source of puzzlement in view of the general opinion that such lesions were not common and Goldberger and Wheeler considered that special circumstances in the diet or environment of their volunteers may have played a part. The lesions themselves suggested their identity with pellagra since they were sharply delimited, dry, scaly and symmetrical.

An investigation of the frequency of scrotal lesions confirms the view that, as judged by the clinical reports, frequency is most variable. It is possible they have frequently been overlooked since they occur on the posterior portions of the scrotum. But it may be that they represent a special deficiency. This is somewhat substantiated by such reports as those of Fitzgerald and Landor and Pallister, described in more detail elsewhere, in which similar scrotal lesions, but which itched severely, were found as the only dermal manifestation of an obviously deficient diet or associated with signs of riboflavin deficiency and nervous disorders, but without the usual skin lesions of pellagra. Spies, however, believes the scrotal lesions part of pellagra.

The mental symptoms of pellagra are manifold and range from insomnia to psychosis. In the mildest cases and in the prodomal period these symptoms are frequently considered to be neurasthenic and McLester, in studying the histories of hospital cases found that a considerable number had been in the institution the year before they were recognized to be suffering from pellagra, and were then diagnosed as neurasthenia. The symptoms complained of are insomnia, anxiety, vertigo, burning sensations, fatigue, palpitation, numbness, backache, distractability and headache. Conduct may be normal but the patient feels unable to work. More severe cases become

melancholic and depressed. Lethargy and stupor are common. Occasionally hallucinations and confusion are seen. The Russian literature speaks of severe cases with fever, delirium and death as "pellagra typhoid."

In describing the early mental disturbances in pellagra Frostig and Spies emphasized the breakdown in personality. Previously brave and robust men become weary and apprehensive. They expect accidents and are pessimistic. Of the other early nervous symptoms of the disease headache resembling migraine is common. It occurs suddenly in the forehead and temples accompanied by scintillating scotomata.

Nervous manifestations include paraesthesias, tremors, muscular cramps, girdle sensations, pain in the neck, burning sensations in the eyes. Pressure pains in pelvis and pain on pressure over the spine occur. A common complaint is that the mouth tastes salty. The Italians sometimes speak of the disease as "Salso." Tremors of the hands and tongue are very common in severe cases and the gait may be paralytic or spastic. Many of these are manifestations of beriberi rather than pellagra but are frequently associated with pellagra.

The frequency and severity of thiamine deficiency in patients with pellagra naturally varies with the prevailing diets. Apparently a great deal of progress is being made now that both vitamins are available in pure form. A frequent observation has been that patients respond strikingly for a time to nicotinic acid therapy and then manifest other symptoms apparently due to an unmasked thiamine deficiency. In many other cases the nervous symptoms of thiamine deficiency appear from the start in combination with the skin lesions of niacin deficiency. A good general rule to follow is to use combined therapy from the start or to add other vitamins if progress seems slow.

Circulatory system changes do not seem to be due to pellagra. Weiss and Wilkins conclude that the changes which are seen are due to thiamine deficiency. The heart is uncomplicated pellagra is small, the blood pressure tends to be low, both systolic and diastolic. Indeed these changes and certain other similarities to Addison's disease have led to the suggestion that pellagra was a chronic disease of the chromaffin and vegetative nervous system.

Mild cases of pellagra in children and cases without dermatitis are exceptionally difficult to identify. The children are undersized and underweight, have little ability to concentrate and progress poorly in school. Sore tongue, indigestion, vomiting and constipation are common symptoms.

More than half of all severe cases of pellagra are anemic. In most cases this is macrocytic and hyperchromic in type but frequently refractory to liver therapy. In a group of pellagrins reported by Spies and Chinn, most of which gave a history of alcoholism, 63 per cent were anemic. Of these,

three-fourths had a color index above 1. Spies and Chinn consider that three factors may operate in pellagra to produce anemia, gastric dysfunction, iron or other deficiencies and hepatic damage causing failure to store the anti-anemia factor. The experiment of Sydenstricker and associates, already mentioned, in which extracts of a pellagrin's liver were found capable of inducing reticulocytosis in Addisonian anemia indicates the latter is not constantly true of pellagra but of course anemia is not constant either. Pre- ... otinic acid deficiency but to a com-

... abnormal in pellagra.

... f the Disease

... aracteristic, and important. Most ... come more and more severe for two ... ve. Such an attack may never be ... to complete cure or vague nervous ... Many more cases recur each Spring ... ck the patient becomes weaker and more emaciated until death occurs, in the average case after five years.

In a few cases the original attack becomes rapidly more severe and gastric and nervous symptoms more prominent. These are the acute cases and death is not infrequent during the first attack.

Pellagra in Infants

Considerable interest attaches to the cases of pellagra in infants. That such occur has been known since the original report by Strambio, in 1794. Snyder investigated the matter in 1912 and was able to report a case in a 2 months' old infant. The mother died of pellagra 2 weeks before the infant developed the rash but the midwife reported that the infant had been emaciated and sickly at birth and had had a profuse, foul-smelling diarrhea since the age of one week. Voegtlin and Harries, who cite these cases, report a remarkable instance in which the infant developed the classical signs of pellagra although the mother remained free of symptoms. Indeed neither the mother nor father had ever had the disease. The infant's earliest symptom was a sore mouth, first noted at 5 months. The following spring diarrhea appeared and the following month (June) erythema of the face, hands and legs. The diagnosis was confirmed by physicians thoroughly familiar with the disease and the skin and mouth lesions were considered typical. Throughout the illness the infant was breast fed. These reports are much like those of beriberi in sucklings in which the mother is usually, but not always, simultaneously suffering from the disease. Pregnancy, and lactation in particular, seem definitely to predispose to pellagra. The incidence of

the disease is very high at such periods. In Voegtlin and Harries' case the mother's milk was analysed and found to be adequate in fats, protein, carbohydrate and minerals, results similar to analyses of the milk of beriberi mothers.

No discussion of pellagra in infancy and childhood would be complete without reference to the view of Goldberger that the disease in our own country is predominantly one of early life, between the ages of 2 and 15 years. Goldberger believed that the hospital records are misleading on this point because of the relative mildness of the disease during childhood.

Atypical Pellagra

Atypical forms of nicotinic acid deficiency mainly fall in one of two groups, nervous disorders and combined deficiency disease. The former group is largely a recent development, the cases having been recognized only through their response to nicotinic acid.

The nervous and mental symptoms of pellagra have already been described. In districts where the disease is common physicians have naturally suspected similar nervous disturbances might be due to latent or early pellagra since, as has been mentioned, nervous symptoms are frequently seen to precede the systemic signs of the disease. Thus Cleckley, Sydenstricker and Geeslin report 19 patients without dermatitis or stomatitis but with nervous symptoms such as are frequently seen in pellagra. Many had sore tongues. All but 4 responded dramatically to nicotinic acid therapy. Musser's experience was quite similar. His patients were anemic, had sore tongues and porphyrinuria as well as nervous symptoms. Bogart observed a similar case the mental condition simulating catatonic schizophrenia. Cure was effected within the week.

Jolliffe, Bowman, Rosenblum and Fein announce that an "encephalopathic syndrome" occasionally seen in the psychiatric wards of Bellevue Hospital and which was heretofore almost invariably fatal responds to nicotinic acid. The syndrome occurs alone or in combination with pellagra or thiamine deficiency disease. It is characterized by clouding of consciousness, cogwheel rigidities of the extremities and uncontrollable grasping and sucking reflexes. Twenty-two cases were treated with large doses of nicotinic acid (1000 mgm. a day plus 100 mgm. of sodium nicotinate given intravenously in dextrose solution and another 100 mgm. sodium nicotinate injected intramuscularly). Fifteen of the cases recovered. The authors suggested that this psychosis represents a result of a complete deficiency and develops before the dermal lesions have time to evolve.

Blankenhorn reports two cases, one resembled hemiplegia, the other diplegia. Both recovered under dietary treatment and the paralysis was ascribed to pellagra.

Pellagra sine pellagra accounts for many other cases of atypical pellagra. Manson-Bahr and Ransford, for example, describe a woman suffering from stomatitis, desquamation of the tongue and chronic diarrhea as such a case. They believe that the cutaneous lesions are less likely to occur in temperate climates.

Castellani proposes the term "dermoberiberi" or pellagroid beriberi to describe patients suffering simultaneously with pellagra and beriberi. The cases he has seen have shown the usual signs of beriberi in the extremities plus roughening of the skin with pigmentation and stomatitis including lesions in the angles of the mouth and keratosis of the hair follicles. The latter, according to the description, seem definitely to be lesions due to vitamin A deficiency and the angular stomatitis is probably the consequence of riboflavin deficiency. The rôle of pellagra in these cases is therefore not prominent.

That beriberi does occur in the presence of outspoken pellagra is evident from the report of Lewy, Himwich, Frostig and Spies. Prompt cure of the nervous symptoms followed treatment with thiamine. These authors believe that the neuropathy of pellagra is actually beriberi and not due to either nicotinic acid or riboflavin deficiency.

The cases described by Wilson as common in Soonchun and Fusan, China, responded to cod liver oil although they appear to have resembled pellagra very closely. The symptoms appeared in the Spring, in most cases recurring year after year. Dermatitis on hands and feet with desquamation and cracking, sore tongue and mouth, difficulty in eating and diarrhea were present. The tongue lesions were those of pellagra. It would appear that other dietary factors than those in cod liver oil must have accounted for the success of Wilson's treatment.

Moore reports that the natives of Nigeria frequently suffer from perleche, sore tongue and genitalia and retrobulbar neuritis. Yeast products are curative. There is little in this description to justify the diagnosis of pellagra. The perleche is now recognized as a feature of riboflavin deficiency and the lesions of the scrotum constitute a rather unusual expression of pellagra as has been pointed out elsewhere.

The cases described by Williams had the mouth lesions of pellagra plus corneal ulceration, nervous irritability and diarrhea. The spleen and liver became enlarged. The place for this syndrome among the deficiency diseases is uncertain. An enriched diet proved to be curative.

Prognosis

The prognosis in severe cases of pellagra has previously been poor. Smith and Stevens collected the records of 520 patients treated by various physicians in California in the period 1928–1935. The mortality rate was 66 per

cent. Among those patients having dementia, diarrhea and oral lesions the rate was 92 per cent. We believe these results to be quite representative.

However, even without nicotinic acid a much better prognosis may be assured by an expert, experienced hospital organization. This has been achieved by circumventing the difficulty in feeding these patients and by giving huge amounts of anti-pellagra foods, yeast and liver extract. Boggs and Padget reporting the results in 102 cases of various degrees of severity, reported a mortality rate of 19.5 per cent. Before Goldberger's work only 26 per cent of a similar group handled in the same hospital recovered. In Voegtlin, Neill and Hunter's very complete studies along similar lines efficient dietary control of the disease among one hundred subjects was conclusively demonstrated. Shortly before the use of nicotinic acid mortality rates as low as 5 per cent were frequently reported. Spies, for example, said the mortality rate in the Lakeside Hospital in Cleveland had been reduced from 54 to 5 per cent by a regimen combining a high calorie diet with yeast, cereal embryo, or liver extract administered parenterally.

Nicotinic acid has further improved these results. Spies, Grant, Stone and McLester followed 321 clinically active cases. Hospitalization was necessary in only 30 whereas previous to the new therapy many more would have required hospital care. Not a single patient died of uncomplicated pellagra. The three deaths which did occur were due to other causes and occurred when the pellagra was improving or had been cured.

Treatment

Nicotinic acid functions as a specific in pellagra but our knowledge of its administration is still incomplete. One thing which seems well established is that the requirement of different patients varies widely. Dosage must be greater during an attack than as a preventive during a remission; it must be greater in severe than mild cases. The requirement varies with the season in persons on a marginal intake and is increased by exercise, infections and the character of the diet. Size and age are factors in establishing the requirement of individual cases. The prophylactic dose of nicotinic acid in depleted individuals varies from 50 to 1000 mgm. a day (Spies, Grant, Stone and McLester).

Acute cases and cases in relapse are best controlled by repeated doses, 50 mgm. ten times in the day (Spies, Grant, Stone and McLester). Large doses should be continued even in the event of toxic manifestations referred to below. One gram daily has been given without harm although most cases may be equally well controlled with much less. Fouts, Helmer, Lepkovsky and Jukes treated 3 cases with 0.5 gram daily and 1 with 1 gram. They considered these doses to be near the maximum. Ruffin and Smith have used 1.5 mgm. per kilo and found it satisfactory. A large number of

patients were observed. This would seem to be near the minimal level of effective medication although the preventive and curative intake in black tongue disease is said to be slightly less.

Dosage has been determined so far both on therapeutic effectiveness and unfavorable reactions. The latter were studied by Sebrell and Butler. Sensations of heat and tingling in the skin occur with large doses. These symptoms occur within 10 minutes of swallowing the dose and persist for 10 to 20 minutes. The peripheral blood vessels are dilated and the blood pressure falls briefly. The skin temperature is increased, circumoral pallor occurs. Often gastro-intestinal hypermotility and even nausea and vomiting follow. Sebrell and Butler determined the dosage level responsible for these symptoms by prolonged tests in adult women. The drug was given orally in 10, 30 and 50 mgm. amounts. Women on the 50 mgm. doses first showed symptoms on the 12th, 16th and 27th day of administration. The symptoms regularly recurred with each dose thereafter and divided doses produced identical although milder reactions. One woman receiving 30 mgm. daily had a reaction on the 32nd day and at times thereafter. None of the women receiving 10 mgm. daily showed the symptoms referred to throughout the test, a period of 3 months. However, others have induced the reaction with 10 and 12 mgm. given intravenously. Evidently the route of administration plays a part. Sebrell and Butler state that reactions are to be expected in some patients receiving 30 mgm. by mouth and in most receiving 50 mgm. Present indications are that these symptoms, while disagreeable, are not dangerous. Toxicity studies in mice and dogs show the fatal doses of nicotinic acid to be very large indeed. Presumably, pellagrins are less prone to these symptoms than normal individuals.

In common with all of the other means of treating pellagra, nicotinic acid is most effective in acute cases. Cleckley, Sydenstricker and Geeslin have emphasized the remarkable effect of such treatment in stuporous cases. The glossitis and acute skin changes respond promptly also. Constitutional and digestive improvement have been frequently noted as extraordinary. The chronic lesions of pellagra respond slowly and the ultimate effect on nervous disorders associated with pellagra is presumably not marked. Whether this represents the usual ineffectiveness of replacement therapy after organic change is well established is not known. Many cases present nervous disturbances due to vitamin B_1 deficiency. This may be treated by supplements of thiamine, the use of which is discussed in another chapter. In the same way the presence of signs of ariboflavinosis, scurvy or other deficiencies may be specifically treated and in any case these vitamins should be provided by supplying an enriched diet. A formula containing 3000 to 4000 calories of which milk is the chief constituent but which also contains

CHAPTER XX

THE VITAMIN B COMPLEX

If Goldberger's diet is fed to men pellagra results. If fed to dogs *tongue*, or canine pellagra, develops. The same diet also causes derm in rats and for many years this was known as "rat pellagra." But it pellagra and many investigators soon became aware of the difference. significance of this rather strange situation, in which the same defic produces different lesions in different kinds of animals, is very great i the case of vitamin B complex where species differences are common. many years the problem was very confusing. It was not solved György succeeded in separating vitamin B_6, now known as pyridoxin, the other B fractions and demonstrating that it was largely responsibl the symptoms of "rat pellagra."

Other B factors have since been isolated until we now have, in additio nicotinic acid, thiamine and riboflavin, a number of lesser B vitam pyridoxin, pantothenic acid, biotin, choline, inositol and folic acid. these the latter two have not been shown to be the cause of human dis *and relatively little is known of their morbid effects in other species.* pyridoxin, pantothenic acid, choline and biotin seem to have consider significance for physicians and the effects of deficiency of these four wil described.

No orderly exposition can hope to be complete for inter-relationships complicated and numerous. A simple example of these relationships is poor absorption of riboflavin during thiamine deficiency. A more comp example is the problem of *imbalance.* Morgan reared dogs on synthe diets and observed sudden and unexpected symptoms when large doses nicotinic acid were administered. "Fortification of foods with those vi mins such as thiamine or nicotinic acid which are available in large qua tities may precipitate conditions worse than the subacute deficiency sta produced by the usual diet *balanced in its inadequacies*" (our italics).

PYRIDOXIN

Pyridoxin (or vitamin B_6) was identified biologically by György in 19 and was isolated and synthesized five years later both here (Harris a Folkers) and abroad (Kühn, Westphal, Wendt and Westphal). It is call *adermin* on the continent. Pyridoxin presumably is identical with factor (Chick and Copping), vitamin H (Booher), factor I (Lepkovsky, Jukes a Krause) and the antidermatitis factor of Hogan and Richardson.

PLATE XXIV Vitamin B_6 deficiency in the rat Effect of the deficiency on the epithelium The upper photograph illustrates the acanthosis, parakeratosis and spongiosis which occurs early in deficiency The lower photograph is from a late lesion which showed thin, pinkish epithelium The pinna is shown with encrusted adherent scale near the tip The remaining epithelium is thin and atrophic, the corium congested, the cartilage irregular

PLATE XXV Vitamin B_6 deficiency An ulcer of the tongue of a rat fed a deficient diet This is a common lesion in B_6 deficiency and one that responds to treatment. The inflammatory response is scanty and there is little evidence of repair although the lesion is of several weeks duration

he lesions in the rat include a rather characteristic dermatitis of the emities (acrodynia) and ears. Lesions resembling sebaceous cysts are mon about the throat and indolent abscesses about the whiskers. ers occur on the tongue. A seborrhoic condition is present generally, earing first over the head. There is no pruritus and alopecia is absent light. The signs appear after at least one month on the deficient diet. he histologic changes have been studied by Antopol and Unna. During early stages the skin of the paws is denuded, edematous and moist, the thickened and scaly, the snout swollen and ulcers common under the gue. The ears are the tissue of choice in following the sequences of ution and repair. The lesion commences with swelling of the epithelial s, widening of the stratum granulosum which is formed of 4 to 5 layers of instead of the normal 1 or 2. Hyperkeratosis is pronounced, the tum lucidum thickened. Intercellular edema and acanthosis occur. the tips of the ears necrosis is sometimes seen, edema of the corium and pervascularization of the supporting structures. The cartilage of the ear fers, the perichondrium loosens and the cartilage becomes disorganized. e edema disappears 24 to 48 hours after a single large dose of pyridoxin. er 3 to 7 days the hyperkeratotic plaques loosen and peel off and the per tissues return to normal. Atrophy of the sebaceous glands also onds to vitamin B_6 therapy.

he curative dose of pyridoxin in rat acrodynia is said to be 5 μ grams mick and Schreffler). Smaller amounts are not uniformly successful. crodynia occurs rather erratically unless egg white (vide infra) be added he diet when the incidence is greatly increased. It is aggravated by weather. Pyridoxin is also a growth factor and plays a role in the zation of dietary fat. Furthermore a number of fatty acids have inct curative effects and Schneider, Steenbock and Platz found that leic acid completely cured acrodynia. Other relationships have been onstrated between pyridoxin, nicotinic acid, riboflavin, thiamine and line. If potassium is withheld from pyridoxin deficient diets myocardial ons are added to the usual signs of acrodynia (Thomas, Mylon and ternitz).

ogs raised on a pyridoxin deficient diet become anemic. The anemia is dominantly microcytic. Polychromatophilia, reticulocytosis and the ence of nucleated red cells are seen. The marrow is hyperplastic and spleen, liver and bone marrow are siderotic. The serum iron is ele-ed These studies, made by Wintrobe, Follis and others, led to the con-ion that pyridoxin deficiency arrests hemoglobin synthesis.

rolonged deficiency in the dog results in severe anemia which does not ond to iron (Street, Cowgill and Zimmerman). The incubation period from 4 to 12 months. After 300 days some dogs became dyspneic and

their hearts were found to be hypertrophied and dilated, chiefly on the right side. Degenerative changes were found in peripheral nerves and the spinal cord. Whether these observations are applicable to cardiac dilatation in thiamine deficiency is not known.

Epileptiform attacks have been observed in pigs and rats as well as in dogs and chicks. Chick and associates state that they appear suddenly. The animals run about in great excitement. They then abruptly fall and pass through stages of tonic and clonic convulsions followed by coma and collapse. Recovery is slow, the animals remaining confused for some time. The fits may last a few minutes or a quarter of an hour. These observations have been confirmed by Wintrobe. Essentially the same behavior was observed in rats.

As a result the vitamin has been used in the treatment of epilepsy as well as other diseases. Jolliffe has reported dramatic relief of the symptoms of Parkinson's disease in particular among cases which had been helpless for less than one year. The rapidity of the response suggested to Spies that vitamin B_6 may exert a sedative effect although the possibility of vitamin deficiency cannot be excluded at this time. Antopol and Schotland secured favorable responses in cases of pseudohypertrophic muscular dystrophy. Kark et al. used B_6 in cases of anemia without response but Bilter and associates induced a slight reticulocytosis in cases of macrocytic anemia.

Spies, Bean and Ashe announce that extreme nervousness, irritability, abdominal pain, weakness and difficulty in walking were observed in several pellagrins after treatment with synthetic preparations, thiamine, riboflavin and nicotinic acid. Very rapid cure of these residual symptoms followed the administration of 50 micrograms of synthetic vitamin B_6.

The inter-relationships between B vitamins may be responsible for the therapeutic effect of pyridoxin in cases of cheilosis. Smith and Martin treated several cases with 20 to 100 mgm. given intravenously and noted prompt response. Machella has had similar results and was led to doubt that riboflavin deficiency is the only nutritional cause of this lesion.

Saturation tests have been described by Spies, Ladisch and Bean who found 8.7 per cent of a 50 mgm. dose given intravenously appeared in the urine within the hour. Three patients fed a pyridoxin deficient diet ceased to excrete measurable amounts of the vitamin. Certain cases of pellagra showed low excretion rates even after treatment. Flexner and Chassin confirm these results in general. They found children between 5 and 15 years of age excrete 21 per cent of a test dose whereas adults under 50 years of age excrete only 8 per cent. The output was 2.5 per cent in patients suffering from post-encephalitic parkinsonism.

Pyridoxin has relatively low toxicity. The lethal dose in rats is 3 gm. per kg. body weight. In prolonged tests at a level of 10 mgm. per kg. body

weight no effect was noted in rats, monkeys or dogs. Spies, Hightower and Hubbard report it is sedative in man.

PANTOTHENIC ACID

When "rat pellagra" is cured by giving pyridoxin, pantothenic acid deficiency becomes manifest. Dermatitis commences in the groin, axilla and on the back between the scapulae. Sores are common about the mouth. The lesion is more generalized than that of pyridoxin deficiency. Interestingly combined pantothenic acid and pyridoxin deficiency in the mouse results in signs of the former while in the rat the latter is dominant. In the mouse the adrenals are not affected but in the rat they become hemorrhagic. Adrenal hemorrhage, one of the most striking effects of pantothenic acid deficiency, was independently discovered by several investigators. György et al. noted it in a third of a series of animals on B deficient diets which had developed panmyelophthysis. Daft and Sebrell were able to separate it from the marrow lesion for under the conditions of their experiments none of the rats with adrenal hemorrhages had lesions of the bone marrow and the one which had an atrophic marrow failed to show adrenal hemorrhage. Daft and Sebrell found hemorrhages in 44 of 72 rats. They also noted that spermatogenesis was reduced.

By further improvements in the diets used Daft, Sebrell, Babcock and Jukes were able to produce adrenal hemorrhages regularly and demonstrate that pantothenic acid was preventive and curative.

György, Goldblatt, Miller and Fulton called attention to the points of similarity between the disease produced in their rats and the Waterhouse-Friderichsen syndrome (purpura, suprarenal hemorrhage, prostration and a rapidly fatal course).

The histological changes in their animals were studied by Ashburn. One of the most striking differences between the adrenals of pantothenic acid deficient and treated animals was their fat content. The depleted animals showed great loss of fat in the zona fasciculata while the treated animals were normal or nearly so. Ashburn points out that since fat is an index of cortical hormone and the animals deficient in pantothenic acid have other signs of cortical adrenal insufficiency, reduced growth, loss of abdominal fat and retarded testicular function the term "hemorrhagic adrenal necrosis" is not satisfactory and represents only one of the manifestations of pantothenic acid deficiency in the adrenal. Skeletal growth was judged in these animals by histologic examination of the epiphyseal cartilage of the tibia. In depleted animals it becomes extremely thin and epiphyseal bone growth is greatly retarded. Under the conditions of the experiment testicular response to treatment was conspicuous only in the number of spermatozoa. Abnormal spermatids were found in both groups. This may have been

due to the relatively brief period of treatment. Chronic zinc chloride poisoning induces the symptoms of pantothenic acid deficiency in rats and can be controlled by pantothenic acid (Gross, Harvalik and Runne). The muzzles of deficient rats are "blood-caked." This is due to a red, fluorescent porphyrin which resembles blood. Removal of the harderian gland prevents its appearance (Figge and Soloman).

The relationship between pantothenate and inositol (as well as other B fractions) has caused many difficulties. Wooley, in studies of B fraction deficiencies in mice, devised a ration which resulted in alopecia of all the body but the head and tail. Both pantothenate and inositol were eventually shown to be involved. By supplying inositol pantothenate deficiency was produced. The symptoms were irritability followed by awkwardness in movement of the hind quarters. Periodic spasms and violent motions were observed verging on convulsions and terminating in more or less complete paralysis. A few days later alopecia appeared on the ventral surfaces, first behind the forelegs on the chest, then over the entire belly and up the sides to the back. Some became completely naked except for the head.

If pantothenate was supplied and inositol withheld the alopecia commenced well up on the thighs. New growth of hair was seen ten days after supplying the missing factor. Both observations are confirmed by Martin and Ansbacher who found that the inositol alopecia did not develop unless paba was given. It (paba) is a dietary factor necessary for the demonstration of the anti-alopecia effect of inositol.

Pantothenic acid deficiency in the chick is marked by incrustations about the eyes, at the corners of the mouth and between the toes. The feathers became inferior and rough. Demyelination is found in the spinal cord and the liver may be fatty (Phillips and Engel). Wintrobe, Miller and others have described the nervous lesions in swine. They consist of degeneration of the peripheral nerves, posterior root ganglia and roots and the posterior columns of the cord. A subacute form of colitis was also found and a retarded ability to heal wounds of the extremities.

Pantothenic acid is also associated with greying of hair. This lesion, nutritional achromotrichia, was first demonstrated in rats by Morgan and Simms as the consequence of 8 to 16 weeks of a B complex deficient diet. The rats showed subnormal growth and their pelage became streaked with grey. The hairs were coarse and lifeless and large, indolent skin ulcers were common. Atrophy was noted in the adrenals, testes, corium of the skin and hair follicles. Greying has now been produced by a variety of diets. Folic acid deficiency also causes greying. And a greater variety of dietary factors seem capable of correcting the condition. Pantothenic acid may be the most active of these but it is not the only one. It was however

PLATE XXVI Nutritional achromotrichia and pantothenic acid The two rats shown in the photograph were fed for 35 days on a diet deficient in B complex but supplemented with nicotinamide, thiamin, riboflavin and vitamin B_6 The animal on the left received a daily dose of 100 micrograms of synthetic dextrorotatory calcium pantothenate as well Both animals were of the same size when the experiment started Nutritional achromotrichia may be due to others factors as well as pantothenic acid but it is obvious that pantothenic acid has prevented greying in this instance Photograph through the courtesy of Dr Klaus Unna

PLATE XXVII. Biotin deficiency in the rat The upper photograph shows the "spectacled eye" lesion formerly considered a sign of "rat pellagra." The lower photograph illustrates the typical position in rat paralysis (Reproduced by permission of Dr. J. J Oleson and the Journal of Biological Chemistry. See Oleson, J. J , Bird, H R., Elvehjem, C. A., and Hart, E. B , J. Biol. Chem., 127: 23, 1939)

the first application of pantothenic acid to medicine but few physicians have had much success in treating greyness with vitamins. Brandaleone, Main and Steele have recently reported distinct results in 2 of 19 elderly patients treated with yeast, pantothenic acid and para-aminobenzoic acid.

It is claimed that pantothenic acid exerts a stimulating effect on gastrointestinal motility (Russell and Nasset).

Pantothenic acid appears to be of very low toxicity. Spies has given 100 mgm. intravenously without harmful effects.

BIOTIN

If rats are fed considerable egg white a characteristic syndrome develops of cessation of growth followed by a desquamating dermatitis which commences in the neck and groin and may extend to become a generalized, exfoliative dermatitis. This lesion can be prevented by feeding vitamin H, or, as it is now called, biotin. The harmful effect of egg white is due to its avidin content which binds biotin and prevents its absorption. Avidin is necessary in the production of the "spectacled eye" lesion because the rat synthesizes biotin. (The same effect can be secured by feeding sulfaguanidine.) The chicken and turkey on the other hand are dependent on an exogenous supply.

Sullivan and Nicholls describe the skin changes in the rat as consisting of hyperkeratosis, parakeratosis, acanthosis and edema, serous cysts and broken hair shafts. The follicles were found to be dilated and filled with keratotic material. The hyperkeratotic scales were laden with sudanophilic fat. A further characteristic of the deficient rats is an awkward gait and stiffened extremities.

These lesions cannot be considered specific and from a histologic point of view discouraging progress has been made in classifying the morphological effects of deficiency of the various members of the B complex in the skin. This has been all the more disappointing because the hope of finding substances useful in dermatology has prompted much of the work with the B fractions and many investigators have sought a relationship between the dermatitis in rats and in man. As long ago as 1929 Findlay and Stern suggested that Pink Disease or acrodynia might be of dietary origin because it resembled the egg white injury lesion. The original name for biotin, vitamin H, was chosen by György from the first letter of *Haut*. Williams, it is true, has described an exfoliative dermatitis in a male patient who had eaten many raw eggs daily for years and whose blood serum biotin content was extremely low. Recovery occurred on ward diet. And Sydenstricker and associates have fed human volunteers a synthetic diet in which 30 per cent of the calories were represented by dried egg white. During the 3rd and 4th weeks a fine, scaly dermatitis was observed which spon-

taneously disappeared. Later some lingual atrophy was noted and the dermatitis recurred. The skin was abnormally dry by the 10th week. Oppel found the human excretion of biotin to be variable. In none of his patients was biotin lacking from the urine. Indeed the total output in urine and feces was from 3 to 6 times as great as the dietary intake, indicating a high level of synthesis.

Caldwell and György report that biotin deficient rats are abnormally susceptible to infection with Trypanosoma lewisi. Possible relationships between biotin and resistance to bacterial infection are suggested by studies which link biotin to lysozyme, an enzyme discovered by Fleming in 1922 and which we refer to again in Chapter XXV. In studies by Meyer and by Laurence it is shown that avidin and lysozyme are closely related if not identical and it is proposed that biotin may serve as the prosthetic group in enzyme systems in which avidin and lysozyme operate. The implications in these observations, readily linked with a wealth of information concerning the nutritive requirements of pathogenic microorganisms which has been gleaned by microbiologic methods of vitamin assay and the realisation of the bacteriostatic effectiveness of bacterial products promises an entirely new field of therapeusis.

CHOLINE DEFICIENCY

The centrifugal growth of the B vitamin field has posed questions of definition. Thus we have been accustomed to think of vitamins as substances which have high biological activity in minute amounts but choline requirements are quite large. Yet choline is generally considered to be a vitamin. It is closely bound to vitamin function; choline deficiency lesions are dependent on an adequate thiamine intake. It has been studied mainly by vitamin techniques and the effects of deficiency follow the general pattern of the avitaminoses. As a vitamin it properly belongs in the B group because of its solubility and distribution.

The initial observation (Best, Hershey and Hunstman) that choline is preventive and curative of one form of fatty liver was an outgrowth of the discovery that de-pancreatized dogs treated with insulin regularly develop fatty livers. It was learned that feeding raw pancreas prevented this lesion. The factor in pancreas which is active (choline) was determined by classical feeding experiments in rats. Best suggested the term "lipotropic." A number of lipotropic substances are now recognized and have been discussed in Section I.

Low choline diets retard the hepatic fat exchange and result in fatty livers. If the deficiency is sufficiently prolonged (in the rat and dog) a degree of interlobular cirrhosis develops (Chaikoff and Connor, Bloomberg and McCollum). If the deficiency is complete and the diet rich in fat the ani-

mals die and their kidneys show massive tubular degeneration and hemorrhage. These lesions were first noted by Griffith who observed further that the thymus was appreciably reduced in size and the spleen slightly enlarged. In the most severely affected rats hemorrhages were also found in the eye. The kidney lesions may be prevented by a choline intake too small to prevent fatty liver. György and Eckhardt noted hemorrhages in the myocardium, adrenal gland, lymph nodes and lungs as well as the kidneys and eye. The hemorrhages in the kidney arise from the arterioles just beneath the capsule.

The close relationship between the B vitamins, choline and the liver is reflected in Rich and Hamilton's experiments in which interlobular cirrhosis was produced in rabbits by diets deficient in choline. Yeast, but none of the pure vitamins, inhibited the development of the cirrhosis. The cirrhotic stage is only the late result of the primary effect of choline deficiency, focal degeneration or necrosis of the parenchyma associated with hemorrhage.

In chickens and turkeys perosis, abnormally thick, short tibial and tarsal bones with dislocation of the joint and displacement of the tendo-calcaneus are the expression of choline deficiency (Jukes). An "x" factor in liver and biotin are also involved in perosis and species differences are pronounced.

Presumably choline deficiency in man, if it does occur, would be most likely to manifest itself by a large, fat laden liver. Since biotin may also cause fatty liver it is worth noting that in experimental circumstances there is a difference betweenthe two in that the biotin liver has increased amounts of both neutral fat and cholesterol while the choline liver is relatively rich in neutral fat. The fatty liver of biotin is not altered by feeding choline.

The absorption of lipotropic substances from the bowel is greatly reduced if pancreatic enzymes are absent and is best corrected by feeding the enzymes.

Best states that while choline deficiency does not influence the development of fatty degeneration due to phosphorous or carbon tetrochloride poisoning it hastens repair. This suggests a useful clinical application.

Some years ago Fields and Wise raised the question of whether riboflavin deficiency collapse, as seen in swine and dogs, might not occur in man and cause death. A feature of riboflavin deficiency is also a fatty liver and the appearance of the liver would be one of the few clues to such a deficiency. The suggestion is given added emphasis by Graham who found many cases of sudden death associated with fatty liver. In the majority of cases death was presumably primarily due to other causes but a residue of cases remained in which no other lesions were found to explain the fatal outcome.

In Elvehjem's review mention is made of the spontaneous occurrence of fatty liver and kidney hemorrhages in premature infants in India.

OTHER MORBID STATES RELATED TO THE

A wide variety of apparently unrelated morbid
vented or cured by administering vitamin B complex
plex. In most instances the active factors are no
reflected in current therapeutics. The use of liver e
conditions other than Addisonian anemia and in m
is clearly beneficial. Not all of these need be review
erence will be made to a number which seem of parti
ise.

VITAMINS AND NEOPLASIA

We have repeatedly emphasized that the avitamin
plicated etiology and pathogenesis. The interde
hormones, the functional balance of various tissues a
sence of exogenous pathogens is becoming steadily
tant. From being exceptional causes of avitaminos
in the Western World they have become the comm
offers great opportunities for the study and better u
esses which at first seem unrelated. A case in point

It has long been thought by some that a relationsh
nutrition and cancer. de Raadt suggested this 15 yea
the incidence and apparent relationship between die
patic cancer in the Orient. He also suspected that die
disposing to cancer in Italy. For many years exper
scanty returns. Kuh's report that massive doses of vi
growth of the Twort mouse cancer was not confirmed
Thatcher). Jorstad's observation that large amoun
peared to assist in the detoxification of certain tar carc
exception to the statement that little progress was m

For not long afterward a very productive series of i
augurated by Kinosita's discovery of butter yellow ca
the rat liver develops following cirrhosis induced by fee
dimethyl-amino-azobenzene, a dye once used in the
Kinosita found that butter yellow only produced cirrh
diet was restricted.

Subsequent work indicated that the characteristic
disposed to cancer was deficiency in riboflavin and a
with casein" and that liver extracts and yeast were co
preventing cancer formation. Rhoads, Dobriner, S
sociates extensively studied the conditions of Kinosita's

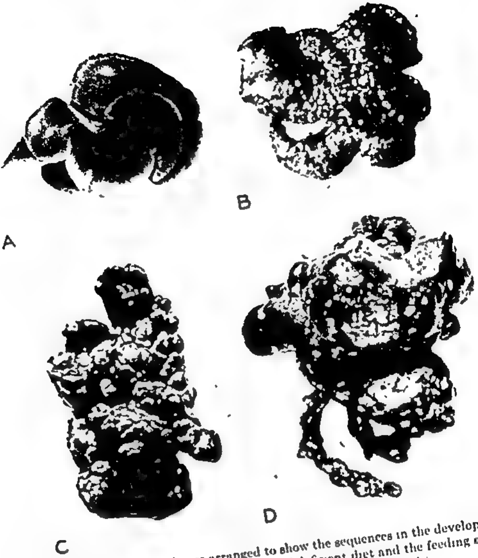

PLATE XXVIII Rat livers arranged to show the sequences in the developmen[illegible] cirrhosis and finally carcinoma as a result of deficient diet and the feeding of bu[illegible] yellow (Photographs through the courtesy of Memorial Hospital)

o destroy the agent and they tested the hypothesis in the case of butter yellow. They succeeded in showing that butter yellow is destroyed in the livers of normal rats and that co-enzyme I is involved in its destruction. Riboflavin contributes to a critical degree in maintaining the [illegible] of the liver and thereby prevents neoplasia. Later studies by du Vigneaud and others have shown that biotin, presumably by [illegible] generation of the rat's liver, can predispose to cancer if butter yellow is given. Thus very suggestive experimental evidence is at hand to support the view that diet plays a considerable role, perhaps determinant role, in the effective detoxification of carcinogenic compounds. No connection can be drawn at this time between these conditions and the known facts of cancer in man.

A further observation links cancer and diet. This is the well recorded fact that patients suffering from carcinoma are usually found to have faulty vitamin functions. We have repeatedly noted cancerous patients whose vitamin C requirements were excessive. In several instances several grams of ascorbic acid daily were required to maintain saturation. Vitamin A plasma values are diminished in individuals with cancer of the stomach and bowel. Abels and associates found low values in 85 per cent of these cases. This was first thought to be an expression of vitamin exhaustion but analyses of the livers of fatal cases showed the vitamin A concentration to be very nearly as great as in the livers of individuals killed in accidents. However liver function tests showed that the cancer patients usually had hepatic dysfunction. It was further shown that while vitamin A supplements afforded little improvement in the plasma concentration the feeding of yeast, choline or lipocaic restored both hepatic function and plasma vitamin A levels.

HEPATIC CIRRHOSIS

Hepatic cirrhosis, of the Laennec variety, follows destruction of the perimeter of the liver lobule by many intoxicants, as for example the butter yellow mentioned in the preceding pages. Considerable protection seems to be afforded the liver cells and therefore considerable influence on the cirrhosis, by diet. Von Glahn and Flinn thoroughly demonstrated the protective action of yeast against cirrhosis induced by lead arsenate. Kensler, Sugiura, Young, Halter and Rhoads have described the changes in the liver following butter yellow administration. Other studies suggest that deficiency of B vitamins or badly constituted diets result in cirrhosis without the [illegible] The studies of György and Gold[illegible] and Hamilton all illustrate this [illegible]nomenon.

Whether these mechanisms operate in man is not proven but is suggested

by two types of evidence; that patients with cirrhosis commonly manifest *symptoms of dietary deficiency* and that dietary treatment during the reversible stages of cirrhosis is very effective in rehabilitating human patients. Patek, for example, reports that 40 per cent of a group of 54 had polyneuritis and macrocytosis and 60 per cent atrophic tongues and "dry skin." He treated these patients with a diet containing 3,500 calories (protein 139, fat 175 and carbohydrate 365) and consisting chiefly of meat, milk, eggs, fruit and green vegetables. Milk was given five times per diem, twice with 25 mgm. dried brewer's yeast. Thiamine chloride was given in daily parenteral doses of 5 mgm. and liver extract twice in the week. Approximately 60 per cent of the group experienced a spontaneous disappearance of ascites. Duration of life was significantly increased as well. A similar regimen yielded equally good results for Fleming and Snell.

If puppies are fed a high casein diet plus thiamine, nicotinic acid, riboflavin, pantothenic acid and pyridoxin growth is somewhat retarded but no external signs of disease appear. If the casein is reduced to 15 per cent anemia, loss of weight and skin ulcers develop and such animals regularly have fatty, cirrhotic livers and often peptic ulcers as well. The lesions are favorably influenced by large amounts of choline or liver and completely cured by choline and liver. Para-aminobenzoic acid and isositol are ineffectual. These studies of Fouts considerably reduce the number of unknowns involved in the problem of cirrhosis and give promise that the active principle will soon be identified.

VITAMIN B COMPLEX AND ESTROGEN

A relationship between estrogen and B complex was ingeniously demononstrated by Biskind and Mark who implanted estrone in rat spleen and noted that it was inactivated during its passage through the portal circulation. If the pellet was moved to a subcutaneous location estrone effects promptly occurred. Estrone effects likewise occurred if the pellet was placed in the spleen and the rat was maintained for more than two weeks on a B complex deficient diet. The phenomenon was believed to be correlative with the butter yellow experiments described earlier in the chapter. If one ovary was transplanted to the spleen and the other removed estrous occurred or did not occur depending on whether the diet was deficient or not.

Clinical application was made of these discoveries by treating women with symptoms of estrogen imbalance with B complex. Biskind, Biskind and Biskind have described 104 patients. They found most of their patients with symptoms of menstrual tension, menorrhagia, metrorrhagia and painful breasts had signs of nutritional deficiency and that women whose chief complaints were due to nutritional deficiency gave histories of abnormal

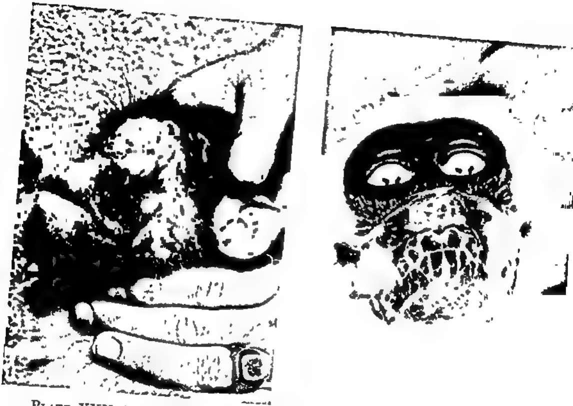

PLATE XXX Lesions of the mouth during dietary deficiency. Two extensive lesions observed by Topping and Fraser in monkeys fed diets deficient in vitamin B complex but supplemented with riboflavin and nicotinic acid. (Reproduced by permission of Dr. N H Topping See Topping, N H., and Fraser, H. F, U S Pub Health Rep, 54 416, 1939)

menstrual functions. Prompt and often dramatic response was secured by giving B complex although in many parenteral administration was required and the dosage was rather large (9–24 mgm. of thiamine, 9 mgm. or more of riboflavin, 60–120 mgm. of niacin amide plus extracts of bran or yeast.) Liver extract was required in some cases. The evidence of nutritional deficiency is not fully reported. In 32 patients cheilosis and atrophic glossitis were found.

ORAL ULCERATION NUTRITIONAL CYTOPENIA

Day, Langston and Shukers described a nutritional disease in rhesus monkeys encountered while studying B complex deficiency. The cardinal signs were leucopenia, neutropenia, anemia and loss of weight. Some animals had thrombocytopenia but this was not marked. Clotting time was not increased. Diarrhea was common and *severe ulceration of the mouth*. The animals died between the 26th and 100th day of deficiency. Riboflavin, nicotinic acid and thiamine did not prevent the lesions. The basal diet was believed to contain adequate amounts of pyridoxin.

A more extensive study of the mouth lesions was made by Topping and Fraser. The histological examinations were made by Tomlinson. These workers separately tested vitamins A and D and found them unimportant to the mouth structures. Deficiency of ascorbic acid resulted in severe gingivitis which only occasionally progressed to gingival necrosis. But of 16 monkeys deficient in B complex 12 developed gingivitis, 10 with generalized necrosis and 3 of the latter progressed to complete, gangrenous necrosis of the cheek (see Plate XXX). Neither riboflavin nor nicotinic acid controlled these lesions which appear to be identical with those described by Langston et al. Thus we have good reason to believe that the B complex is closely related to the maintenance of the gingival and oral tissues.

Additional, confirmatory evidence comes from the studies of Becks, Wainwright and Morgan. Dogs were used in long term experiments primarily designed to determine the role of nicotinic acid in the production of mouth lesions. The paradental lesions caused by prolonged nicotinic acid deficiency are described in Chapter XIX. The epithelial erosion of canine black tongue was not reproduced by either complete or partial nicotinic acid deficiency. Deficiency of filtrate factor on the other hand caused just such extreme necrotic lesions as noted by Langston. The supporting structures of the teeth were frequently laid bare and the alveolar bone was sometimes porous and atrophic. These lesions could be largely controlled by supplements of pantothenic acid but other filtrate factors were not tested. Yeast filtrate gave complete protection. Presumably more than pantothenic acid is required for complete protection.

CONGENITAL MALFORMATIONS

In discussing the effects of vitamin A deficiency on the organs of reproduction a number of studies were referred to in which congenital malformations followed vitamin deprivation of the mother. This is hardly surprising in view of the exacting requirements of embryonic tissues. It is, on the other hand, a very hopeful development in a field where there appeared to be little that could be done.

A series of reports by Warkany and his associates have greatly supplemented the knowledge already referred to. Using rats fed a diet of corn meal, 76; wheat gluten, 20; chemically pure calcium carbonate, 3, and sodium chloride, 1; Warkany has produced a great variety of congenital malformations in 39 per cent of 484 offspring. Malformations were more common in the 2nd and later litters than in the first. The lesions found consisted of receding mandibles, fusion of certain ribs, shortened forelegs and cleft palate. An adequate stock diet or a supplement of liver prevented all the lesions. Sections of the tibia from representative animals have been reproduced in Plate XXXI.

The cleft palates were of the post-alveolar type. They were also induced, along with the other malformations, by a diet of sucrose, casein, vegetable oil and salt mixture supplemented with thiamine, pyridoxine, calcium pantothenate, nicotinamide and choline. Vitamins A and D were supplied separately. The lesions were ascribed to riboflavin deficiency.

A somewhat different pattern of defects was produced by a rachitogenic diet but this can best be interpreted as fetal rickets. Cartilage overgrowth and bending of the long bones was conspicuous.

PANMYELOPHTHYSIS

A striking complication in certain rats on a B complex deficient diet supplemented with thiamine and riboflavin is panmyelphthysis, described by György, Goldblatt, Miller and Fulton. The lesion was associated with the substitution of rice starch in the basal diet by cane sugar but was prevented by using Peter's eluate, a crude B_6 concentrate. More purified preparations of pyridoxin were inactive. Their observations were based on 72 rats which showed the syndrome the signs of which were similar to those of aplastic anemia involving agranulocytopenia, thrombocytopenia and erythrocytemia—thus the suggested term panmyelophthysis. The disturbance seemed analogous to the anemia already described in monkeys which was discovered by Day, Langston and Shukers and the anemia in dogs studied by Miller and Rhoads although more severe. This may in part have been due to more effective supplementing of the basal diet.

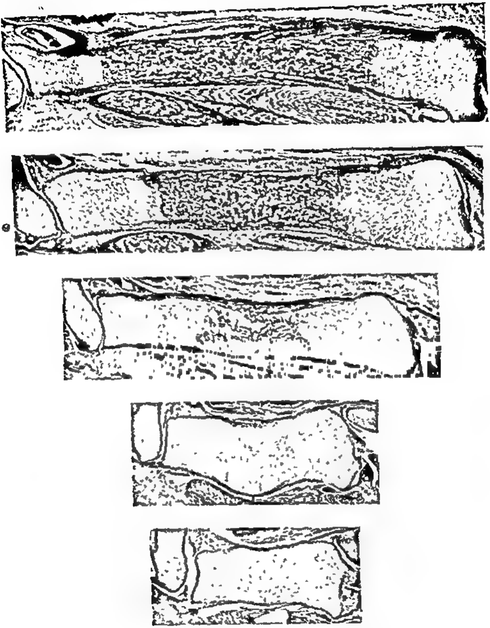

PLATE XXXI Effect of maternal malnutrition on skeletal tissues of young Tibia of rats showing various degrees of shortening, retarded ossification and abnormal cartilage growth The upper photograph is a normal tibia, the others in sequence more and more extreme lesions These defects are preventable by adding small amounts of liver or riboflavin to the maternal diet (Courtesy of Dr Joseph Warkany)

PLATE XXXII Purpura, a striking, late manifestation of deficiency in rats wit panmyelophthysis. (Reproduced by permission of the Rockefeller Institute fc Medical Research and the authors, P Gyorgy, H. Goldblatt, F. R Miller and R. F Fulton, J Exper Med, 66. 579, 1937.)

MACROCYTIC ANEMIA

has been widely accepted for some years that the "extrinsic factor" of le is commonly associated with the vitamin B complex, as, for exle, in yeast. Wintrobe, in reviewing the reports of the use of exic factor finds that roughly one-third of the cases of Addisonian anemia ond to treatment with extrinsic factor. In 15 thoroughly studied cases Vintrobe brewer's yeast gave maximal response in 5 patients who red 1 to 2 grams per kilogram daily, moderate response in 2 and slight onse in 3. The latter cases were but slightly responsive to oral liver. ly 1 patient was liver (orally) more effective than yeast.

rather close counterpart of Addisonian anemia, but one directly due to of extrinsic factor has been extensively studied by Wills. Her attitude esent towards the nutritional agent involved has been stated in a study Vills, Clutterbuck and Evans. Experimental macrocytic anemia (in monkey) they conclude, is not due solely to a deficiency of Castle's exic factor for their tests show a lack of parallelism between the effects of ous agents in the two conditions. Multiple deficiency may be involved. hoads' studies have shown that Goldberger's pellagra producing diets apable of inducing in swine a disease remarkably similar to Addisonian nia, including neurologic disturbances, achlorhydria and stomatitis. It is evident that the vitamin B complex is not only of great importance source of extrinsic factor but possibly also as a cause of those stigmata iated with lack of intrinsic factor.

stle et al. have demonstrated that the joint administration of purified n, thiamine, riboflavin, niacin, pyridoxin, pantothenate, paba, choline, tol, biotin, xanthopterin and folic acid is incapable of inducing remisin Addisonian anemia. These factors do not constitute the extrinsic r. It is believed, however, that the extrinsic substance is a fraction e vitamin B complex, a factor still unidentified.

PABA AS A SULFONATE ANTAGONIST

ra-aminobenzoic acid is routinely used in cultivating pathogens when namides have been administered to the patient. This is an extremely l application of nutrition and one with which physicians are generally ainted. *Henry's recent review is recommended.* It seems approe here to point out simply that the action of PABA as a sulfonamide gonist is apparently dependent on the nutritional requirements of the eria. Thus Woods and Fildes explanation was that sulfonamides funcby interfering with an essential metabolite (para-aminobenzoic acid), hibitor being so closely related to the metabolite that it can fit the same ne but sufficiently unrelated as to be an inadequate substitute from oint of view of the pathogen. The distribution of sulfonamide-antag-

onists suggests that PABA is of predominant importance in most cases i which such phenomena have been seen.

In addition to the immediate, practical importance of this fascinatin relationship there is the implication, hardly explored as yet, that nutritio may be a two-edged sword in resistance to infectious diseases (see pages 34 to 352).

Chapter XXI

VITAMIN C DEFICIENCY

The conquest of vitamin C deficiency, or scurvy, is one of the dramatic chapters of the history of medicine. It appears to be a chapter which is largely completed for although the requirements of vitamin C are not definitely known and certain etiologic factors must yet be explained the practical significance of scurvy has largely disappeared excepting where it develops during the course of other diseases.

HISTORICAL

A few references to the history of scurvy or scorbutus should prove of interest. Hippocrates and Pliny both wrote of it and cases occurred in the armies of Caesar Germanicus and Aelius Gallus. It has plagued commanders and medical officers ever since. It ravaged the troops of Napoleon in Egypt. During the Civil War 46,910 cases and 771 deaths were reported in the Union Army. At a post near Council Bluffs, Iowa, half the force was invalided with scurvy and 30 per cent died during 1821. In the First World War epidemics were common both in the field and in prison camps. German troops endured 23 epidemics on the Eastern Front and 1 on the Western. In 1915, during the defense of Kut-el-Almara a mixed army of Indian and British troops were on reduced rations and the British developed beriberi, the Indians scurvy. The protective food which the British ate was horseflesh. By the time the army capitulated in April 1,100 cases of severe scurvy had occurred among 9000 men.

From the time of Vasco da Gama through much of the 19th century scurvy was a plague to sailors Vasco da Gama's force of 160 men was reduced to 60 by the scurvy. Magellan's crew was decimated. Those who escaped probably did so by eating the ship's rats which commanded half a ducat. Jacques Cartier lost 25 men from scurvy. "An unknowen sicknes began to spread itselfe amongst us after the strangest sort that ever was eyther heard of or seene, insomuch as some did lose their strength, and could not stand on their feete, then did their legges swel, their sinnowes shrinke as black as any cole. Others also had all their skins spotted with spots of blood of a purple colour; then did it ascend up to their ankels, thighes, shoulders, armes and necke; their mouth became stincking, their gummes so rotten that all the flesh did fall off, even to the rootes of the teeth which did also almost all fall out."

Many like experiences have been described by Vogel. Correction came largely from the work of three great naval hygienists, James Lind, Sir Gilbert

Blane and Thomas Trotter, backed by the practical experience of James Cook. Lind's "Treatise of the Scurvy" (1753) was the first authoritative account of the disease. It is worth noting that he believed the disease to be due to a combination of factors of which the lack of fresh vegetables was predominant. Cold and dampness Lind believed to be important for the warrant officers who had the same fare as the men below decks but whose quarters were warm and dry were usually spared. It was Lind who in 1747 took 12 scurvy patients and kept them on the same diet but used 6 different supplements, a quart of cider, elixir of vitriol, vinegar, sea water, a garlic, mustard electuary and oranges. In 6 days the lucky ones who received the oranges were well enough that one returned to duty and the other was assigned to nurse his companions.

James Cook was phenomenally successful in maintaining the health of his crews. When the *Resolution* returned from more than three years in the Pacific only one man had died. Cook relied on good drinking water, cleanliness, dry quarters and clothes and vegetables whenever available. Malt wort and sauerkraut were used as dietary supplements. In remarking on Cook's demonstration Oliver Wendell Holmes wrote that medicine had "learned from a sailor how to keep off scurvy."

The first description of scurvy by a physician, according to Lind, was in a letter by Ecthius to Dr. Blienburchius of Utrecht (1541). Balduino Ronsseus, in a volume devoted to scurvy and written in 1564, referred to the use of scurvy grass, water cress and oranges.

The characteristic of the vitamin that proved the best clue to its nature was its instability. This was shown by the work of Zilva in England, by Vedder and by King in America and by Bezssonoff in France. In their attempts to isolate the vitamin from lemon juice or cabbage it became increasingly evident that oxidation rapidly destroyed the potency of the vitamin sources.

This was further confirmed by studies of the methods of preserving antiscorbutic foodstuffs. The commercial canning process (Eddy and Kohman, 1924–5) was found to owe its protective action against loss of vitamin C potency to control of oxidation. In 1922, Zilva showed that decitrated lemon juice lost 80 per cent of its potency in one-half hour if made N/20 alkaline and exposed to air at room temperature. No loss of potency occurred if air was exlcuded. This was confirmed by Kennedy in Sherman's laboratory in 1926.

These and many other similar studies proved that vitamin C (the antiscorbutic factor) is an easily oxidized compound and one whose oxidation is notably reduced by the maintenance of an acid reaction. For reviews of these studies see King.

Progress toward isolation of the vitamin was delayed from 1916 to 1920

by the infection theory of scurvy origin. In 1916, Jackson and Moore recovered from guinea pigs made scorbutic by a diet of oats and milk, a diplococcus which they suggested might be the etiological factor. Since oats and milk were known to provide a complete diet for rats Jackson's results seemed to exclude diet as a causative factor.

The following year, McCollum and Pitz confirmed the production of scurvy in guinea pigs by feeding diets of oats and milk and supported Jackson's theory. McCollum has described their attitude at the time in these words: "They found it difficult to believe that the disease could be due to the lack of a specific substance, for milk alone suffices as the sole food for all young mammals during a critical period of their lives."

Examination of their oats and milk fed guinea pigs showed the caecum distended with impacted feces. They felt that this also confirmed the infection theory.

Shortly after, however, Chick and Hume and Cohen and Mendel produced evidence that reconfirmed the probable dietary origin of the disease. Chick and Hume showed that milk was a far poorer protective against scurvy than had been assumed and Cohen and Mendel by feeding a superior diet were able to produce scurvy in a guinea pig without developing impacted feces or producing caecal lesions.

A few years later Parsons furnished the final explanation of McCollum's inconsistent results. Parsons found that while oats and milk constituted a protective diet for rats this was due, not to the vitamin content of milk but to the complete immunity of the rat to scurvy. Parsons found that the livers of rats reared on a diet deficient of the antiscorbutic factor contained significant amounts of this factor and that rats, unlike guinea pigs and man, synthesize their needs of the vitamin. This was confirmed by further studies of Parsons and Hutton and by Lepkovsky and Nelson.

The false trail was therefore abandoned and search for the antiscorbutic substance resumed.

A specially effective aid to this search was found in significant contributions made by Tillmans and Hirsch. These chemists, at Frankfurt, Germany, had occasion to distinguish between fresh and stale and between true and artificial fruit juices. They found that distinction could be made by using an oxidation-reduction indicator known as phenol-indo-phenol. Fresh juice gave a strong reaction with the reagent, stale juices a lesser reaction. Artificial juices did not affect the indicator.

Zilva showed also that antiscorbutic juices bleach phenol-indo-phenol. Zilva and his associates found that they could determine the reducing capacity of antiscorbutic potent substances by means of the indicator but that the results did not always parallel estimation of vitamin C by animal tests. From these comparisons Zilva reached the conclusion that

vitámin C itself did not reduce indophenol, but that the decolorization of the indicator was due to a reducing substance closely associated with the active principle, which tended to prevent oxidation.

To this view of Zilva's, Tillmans took exception and contended that it was the vitamin itself which bleached the indicator. In advancing this view he relied on studies that produced evidence that the reduction of the indicator was due to vitamin C itself, that the indicator measured the concentration of the vitamin and its physiological potency, and that the vitamin might be hexuronic acid. He held that the oxidation of the vitamin was reversible and that in the first stage of oxidation the vitamin was more prone to destruction by further oxidation than in its original reduced form.

Though Tillmans did not know of it at the time, Szent-Györgyi had already demonstrated the reversible oxidation of hexuronic acid. The further resemblance of the vitamin to hexuronic acid developed a little later.

In 1932, Waugh and King precipitated from lemon juice an actively antiscorbutic substance which they were able to isolate in crystalline form. They reported that it appeared identical with the hexuronic acid Szent-Gyorgyi had recovered from adrenal cortex, oranges and cabbage. Waugh and King share the credit for the crucial work that identified vitamin C as a hexuronic acid.

Szent-Gyorgyi's part in this development may be summarized as follows: In 1928, he isolated from adrenal cortex a highly reducing hexose derivative, hexuronic acid. He noted at the time that the compound decolorized the phenol-indo-phenol reagent and this led him to its possible identity with the reducing substance postulated by Zilva. In 1932, the year in which Waugh and King reported their results, Svirbely and Szent-Györgyi furnished further evidence of the identity of hexuronic acid and vitamin C.

The structure of hexuronic acid was established by Haworth and Hirst and collaborators and in the same year, Reichstein, Grussner and Oppenauer synthesized it.

The term hexuronic acid has been superseded by the name ascorbic, now universally adopted to designate the pure vitamin.

ASCORBIC ACID REQUIREMENTS

The concentration of ascorbic acid in the blood and the excretion in the urine are easily measured and rapidly reflect the amounts ingested. Thus vitamin C balance is simple to study. It has been very thoroughly investigated. From our own experience and an examination of the results of others it appears reasonable to draw the following conclusions. The values all refer to adults.

Clinical manifestations of scurvy are prevented by a per diem intake of

15 to 20 mgm. If the validity of the capillary fragility test be accepted as a criterion of mild scurvy, the daily intake should be set at between 20 and 30 mgm. (Göthlin).

An intake of 50 mgm. is sufficient to elevate the blood concentration in average adults. This was considered optimal intake some years ago.

The present optimal level is estimated to be approximately 100 mgm. on the basis of studies which show that this is enough to maintain saturation. Amounts in excess of 100 mgm. are largely lost in the urine unless the individual has been delpleted.

In explaining the problems faced by the Committee on Foods and Nutrition of the National Research Council Roberts points out that while the amount needed to maintain blood levels is known as well as the greater amount required for saturation it is not known what a normal blood level is or whether saturation is necessary or even desirable. The Committee therefore took a compromise position and recommended 75 mgm. for adults, 100 mgm. during preganey, 150 mgm. during lactation and a scale of from 30 mgm. for infants under one year to 80 mgm. for adolescent children.

The problem is little different than that of the requirements of other vitamins. In the case of ascorbic acid, however, there is very clear-cut proof that in experimental animals an intake twice or three times that required to prevent clinical signs is necessary to prevent anatomical stigmata in certain organs And from general biological principles, the concentration of vitamin C in human milk, the concentration in certain organs and the ready availability of large amounts of vitamin C in foods it would seem that the Committee's recommendations are reasonable and justified.

In individuals the problem is different. It is worth noting that irregularities in excretion, blood levels and all other measurements emphasize in the case of vitamin C the wide range of individual variations. These, however, are best discussed as factors which influence the occurrence of scurvy. They have been recognized by clinicians for many years.

ETIOLOGY OF SCURVY

The opinion of pediatricians twenty years ago may be gathered from two quotations. In 1917 Hess wrote: "Infantile scurvy is not, however, a simple dietary diease. The diet is at fault in allowing the intestinal bacteria to elaborate toxins It is doubtful whether the toxin is always the same and therefore whether strictly, from an etiologic viewpoint, this disorder should be regarded as an entity." In one of his last contributions to the subject he stated that he was still in accord with this view. Brennemann, in 1923, expressed the opinion: "Scurvy occurs only in children

with a predisposition to the disease—and the individual factor may play a larger part than we now know." These views were based on observations of discrepancies between diet and the occurrence of scurvy. On the same intake not all individuals developed the disease. Scurvy sometimes appeared and disappeared although the diet was not changed. On the same diet one group of patients developed scurvy and another neuritis (Darling).

Is there any evidence that scurvy is more complicated than the general opinion holds? Is there reason to believe that these earlier observations were due to phenomena we now neglect and not simply to the cruder methods of study then available?

A discrepancy seems to exist between the calculated requirement and the occurrence of scurvy among certain groups. Stefansson has pointed out what a tremendous gulf this is. By the calculations of nutritionists primitive peoples should all be invalided by scurvy, he writes, whereas actually they are robust and healthy. He concludes that one of several errors exists in our present conceptions. Too much reliance has been placed on guinea pig experiments which are not applicable to man, the estimated requirements are 2 to 10 times too great, the ability of man to utilize and extract vitamin C from foodstuffs has been underestimated, the effect of cooking overestimated or there exists in animal tissues a component other than ascorbic acid which is capable of preventing scurvy. Possibly man can adapt to a low vitamin intake.

In guinea pig experiments reported in 1931 (Dalldorf) it was pointed out that animals placed on a vitamin C free diet developed capillary fragility but that this did not develop regularly but was subject to a transient reversal during the early stages of the depletion. In other words processes of adjustment or compensation were probably present. This view has since been confirmed. Giroud speaks of *adaptation* to explain the interesting observation that on a depletion diet the organ vitamin stores fall and then rise slowly so that they are greater at the end of the first month than at the 15th day. Wachholder had a similar experience. On a C free diet the stored vitamin was rapidly lost during the first 3 days (this period corresponds to the initial rapid fall in capillary resistance). From the 3rd week on the values of the intestine, lung and heart remain practically constant until death, while the liver, skeletal muscle and plasma show a transient increase. The capacity of various tissues to oxidize vitamin C and to reduce dehydro-ascorbic acid undergoes marked variations, being increased shortly before symptoms of scurvy appear and much reduced when the symptoms are fully developed. Wachholder observed the same mechanism in human cases. A deficiency of 300 to 500 mgm. is well counterbalanced. Only a deficit greater than 1000 mgm.

produces symptoms. Baucke's studies of the oxygen consumption seem to indicate the same mechanism. While consumption is greatly diminished during the initial depletion period it increases for a time to greater than normal values shortly before the onset of symptoms.

Mouriquand has also written of this matter. In a recent study by Mouriquand, Edel, Dauvergne and Lavaud it is pointed out that guinea pigs, when they begin to lose weight show a remission of symptoms. Symptoms could be retarded and minimized by withholding water. On a regimen designed to limit the fluid intake guinea pigs survived for from 30 to 40 days on a C free diet. This may be due to reduced rate of glomerular filtration. The authors state that macroscopic evidence of scurvy was lacking at post mortem examination.

Whatever the mechanisms are which cause one individual to differ from another in ascorbic acid requirement or utilization they have a very practical significance as any group of cases will show. A typical example of this individualism was reported by Elmby and Warburg. Of 29 cases of mild scurvy (hemorrhagic diathesis plus low blood plasma vitamin values) 26 responded within 10 days to 300 mgm. ascorbic acid given orally. In all 26 the symptoms disappeared and the plasma value became normal. The other 3 showed no improvement in either respect. They were therefore given 300 mgm. parenterally on each of ten days and still failed to respond. Treatment was thereupon changed to the juice of 10 lemons daily. Under this regimen the blood values promptly rose and the symptoms disappeared. Elmby and Warburg suggested that another factor in the lemon juice was essential, in these particular cases, to utilization. Whatever the explanation the experience is not an unusual one.

Possibly the value of the diet in other factors modifies the vitamin C requirement. In the discussion of the incubation period of scurvy which follows reference is made to the recent reports in which individuals on a varied and ample basal diet survived for many months without ascorbic acid and without signs of scurvy. The epidemics of the disease have without exception been characterized by diets deficient in many factors other than vitamin C alone. These observations have led certain persons to suggest that man is capable of synthesizing ascorbic acid to a certain degree or that he can utilize small amounts more efficiently if his intake is small. It would seem more in harmony with natural laws if the difference between species in respect to vitamin C, in which rats are immune and man and guinea pigs susceptible, were relative rather than absolute.

However these are purely speculative. Enough has been said to illustrate the problems which still exist. While they are not important in a consideration of ascorbic acid nutrition as it applies to the population as a

whole they are doubtless of considerable significance in understanding the erratic occurrence of the spontaneous cases of the disease and therefore of particular interest to physicians.

It would be incorrect to infer that these secondary factors can do more than modify the development of scurvy. Some years ago when volunteers were first enduring ascorbic acid deficient diets (see Incubation Period) without developing clinical signs of disease there was a great deal of surprise and a rather critical re-examination of the question of the etiology of scurvy. The issue was largely settled by the classical experiment of Crandon (see Crandon and Lund) who lived on a vitamin C deficient diet for more than 6 months. After 134 days hyperkeratotic papules developed over the buttocks and thighs. Four weeks later perifollicular petechiae appeared, particularly after standing for long periods, the gums became boggy and fatigue became extreme. There can be no doubt, therefore, of the genuineness of scurvy due to a deficiency of vitamin C alone although the long incubation period and absence of more severe lesions suggests that predisposing factors were lacking. Others have assumed that the differences between the signs of deficiency in Crandon and in classical scurvy were due to the fact that the latter are multiple deficiencies.

THE ANATOMIC CONSEQUENCES OF VITAMIN C DEFICIENCY IN EXPERIMENTAL ANIMALS

The morbid effects of vitamin C deficiency are best studied in experimental animals where complete dietary control permits us to examine the lesions at all stages of the disease. We have therefore chosen to describe the pathology of experimental scurvy before we discuss the lesions in human cases. In both cases the lesions are, for all practical purposes, the same and it is interesting to recall that the commonly accepted explanation of the pathogenises of scurvy, while established in controlled, experimental material, has only confirmed the theory of Aschoff and Koch which was based on human cases of the disease.

Aschoff and Koch characterized scurvy as a condition in which defective intercellular materials were formed. Wolbach and Howe substantiated this theory by demonstrating that in complete deprivation of vitamin C certain skeletal tissues formed a fluid material rather than their natural products, dentine or bone. The physical character of the fluid may be demonstrated by administering the vitamin which causes it to rapidly solidify or gel. The vitamin is therefore an essential ingredient in certain intercellular materials While this explanation is not generally accepted as yet it is in conformity with our own experience.

The alternative explanation of the scorbutic lesion is that deficiency

produces an atrophy of the skeletal tissue cells. This view was warmly defended by Höjer both on the basis of the minute structure of the lesions and on other observations among which were the decreased oxygen consumption, reduced gastric acidity, diminished adrenalin content of adrenal glands, retarded hematopoesis, decreased opsonic index and resistance to infection. To proponents of the "jellation" theory these are ascribed to secondary changes. The phenomena of complete deficiency and repair have not been satisfactorily explained on the basis of cellular atrophy.

The most recent contribution to the pathogenesis of scurvy is the work of Ham and Elliott who also object to the "jellation" theory and conceive of the scorbutic process as an atrophy. They found no histological evidence that either excess fluid was present during scurvy or that the rate of repair was more rapid than occurs normally in the healing of fractures. It is very likely that the issue cannot be settled by histological means. The reasons given by Ham and Elliott do not seem conclusive. A point which seems to favor the acellular character of the phenomena of acute scurvy is the generally accepted evidence that the formation of collagen proceeds without the immediate intervention of cells. This view was expressed by Hertzler who said: "My researches have convinced me that the cell is not primarily the active agent, but that the initial processes are chemical and are identical with those of blood coagulation, the cell playing an entirely secondary role." Baitsell also believed he had observed collagen formation in fibrin clots in the absence of cells. Since ascorbic acid is of cardinal importance in the formation of collagen this evidence would seem to be further substantiation of the view that the essential phenomena of complete deprivation are intercellular. Most investigators who have taken exception to this view have been dealing with partial degrees of deficiency.

Other techniques may be necessary to settle this problem. von Jeney and Törö, who grew fibroblasts in tissue cultures, report that ascorbic acid controls the formation of fibrils in the ground substance. More satisfactory conditions might be secured if collagen formation were studied free from cells. Laue patterns indicate that collagen is the result of crystallization in a sol. Such experiments are therefore possible and might well yield a decisive answer.

Experimental scurvy has been extensively studied by Glasunow who characterized it as a loss of the ability of differentiation by the mesenchyme. Studies of the healing of wounds, as reported by Mazoue and Randoin fully describe the effect of depletion on the formation of collagen and also reveal that the histiocytic and giant cell response to experimental granulomata is interfered with.

The guinea pig is the animal of choice in studies of scurvy, being very

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The most recent contribution to the pathogenesis of scurvy is the work of Ham and Elliott who also object to the "jellation" theory and conceive of the scorbutic process as an atrophy. They found no histological evidence that either excess fluid was present during scurvy or that the rate of repair was more rapid than occurs normally in the healing of fractures. It is very likely that the issue cannot be settled by histological means. The reasons given by Ham and Elliott do not seem conclusive. A point which seems to favor the acellular character of the phenomena of acute scurvy is the generally accepted evidence that the formation of collagen proceeds without the immediate intervention of cells. This view was expressed by Hertzler who said: "My researches have convinced me that the cell is not primarily the active agent, but that the initial processes are chemical and are identical with those of blood coagulation, the cell playing an entirely secondary role." Baitsell also believed he had observed collagen formation in fibrin clots in the absence of cells. Since ascorbic acid is of cardinal importance in the formation of collagen this evidence would seem to be further substantiation of the view that the essential phenomena of complete deprivation are intercellular. Most investigators who have taken exception to this view have been dealing with partial degrees of deficiency.

Other techniques may be necessary to settle this problem. von Jeney and Törö, who grew fibroblasts in tissue cultures, report that ascorbic acid controls the formation of fibrils in the ground substance. More satisfactory conditions might be secured if collagen formation were studied free from cells. Laue patterns indicate that collagen is the result of crystallization in a sol. Such experiments are therefore possible and might well yield a decisive answer.

Experimental scurvy has been extensively studied by Glasunow who characterized it as a loss of the ability of differentiation by the mesenchyme. Studies of the healing of wounds, as reported by Mazoue and Randoin fully describe the effect of depletion on the formation of collagen and also reveal that the histiocytic and giant cell response to experimental granulomata is interfered with

The guinea pig is the animal of choice in studies of scurvy, being very

susceptible to deficiency of the vitamin. Rats, swine, fowl and calves are evidently immune. Many studies of the effect of vitamin C deficiency in the rat have been published.

"Rat scurvy" was first reported many years ago by Shipley, McCollum and Simmonds. The diagnosis was not proven. However Kollath, some years later, believed he had produced scurvy in rats by feeding a B deficient diet in which peanut oil was substituted for the cottonseed oil. One of his rats was diagnosed by Schmorl as a case of scurvy but the report is not given and the question has remained unsettled. An explanation of this curious state of affairs may lie in the observations of Muselin, Tully, Longnecker and King on the effect of inanition, various lipids, etc., on the excretion of vitamin C by the rat. Vedder and Rosenberg also report that large doses of a vitamin A rich oil caused scurvy like symptoms in rats and that 0.5 mgm. of ascorbic acid protected the animals "almost fully." The possibility of producing scurvy in rats, fully immune to simple deprivation, is a very interesting one and might prove a useful tool in studying morbid mechanisms capable of producing scurvy. However these observations do not establish scurvy in the rat and until clear cut histologic evidence is submitted the problem cannot be settled. The same is true of the other animals occasionally reported as scorbutic. Hjärre and Lilleengen found waxy degeneration in the muscles of young calves. This lesion is said to be relatively common during late winter and spring and in years of poor harvest in Sweden, Germany and France. They claimed to have observed scorbutic lesions in the bones and teeth of such animals and to have produced similar lesions in very young calves. It may well be, therefore, that immunity and susceptibility to scurvy are relative rather than absolute. The "scurvy-like" disease of chicks (Dam) was soon shown to be due to deficiency of vitamin K.

It will be remembered that much earlier in the study of the avitaminoses (1918) Harden and Zilva found rats grew better if ascorbic acid was supplied and that Drummond found their litters were larger. At that time Drummond wrote of the rat's requirement: "they are sufficiently well marked to dispel any idea that there exists a fundamental difference in the nutritive requirement of the two types of animals" (rats and guinea pigs).

Scurvy in the Guinea Pig

In sharp contrast to the rat the guinea pig rapidly develops severe and fatal scurvy. The first manifestations of the disease seem to be capillary fragility which is promptly followed by microscopic lesions in the teeth. Wasting develops rapidly within the second week and most animals die at the end of the third week of completely deficient diets. On partially

deficient diets the animals live for months and develop the lesions we have long associated with scurvy in man.

The lesions pathognomonic of scurvy occur in the teeth and long bones. In these structures the parenchymal cells, whether odontoblasts or osteoblasts form fluid instead of the normal substantial dentine or bone, or, in cases of partial deprivation they form defective materials which, while solid, are distinctly inferior. A definite law seems to govern the type of degradation product of these cells. On a moderately deficient diet the most highly specialised cells, the odontoblasts, form bone, but if the dietary deficiency is more pronounced they revert to simple connective tissue cells. The osteoblasts, less highly differentiated, form an inferior bone or collagen depending on the degree of deficiency.

The result of this fundamental change is that teeth and bones cease to grow, and become gradually more porotic and fragile. Thin bones are, accordingly, characteristic of both experimental and human scurvy. Other sequelae of these changes are weakening of the costo-chondral junctions and epiphyses, pathological fractures and separation of the periosteum at the sites of muscle insertions and hemorrhage beneath the periosteum.

The fascia and ligaments are likewise weakened. Todd mentions this. "In certain unpublished studies of vitamin C deprivation in guinea pigs Dr. Milton B. Cohen and I have found such weakening of ligaments that it is difficult to skin the animal without tearing the ligaments of joints particularly those of the knee and cervical vertebral column. The head of the animal indeed is very apt to be torn from the vertebral column by that ordinary force necessary to draw the skin from the cranium."

Skeletal Lesions

The first effect of deprivation in the bones is best observed at their growing ends. Here the active osteoblasts are seen, within the first weeks of the deficiency, to lose their round or oval shape and become fusiform. They migrate away from the trabeculae and are indistinguishable from fibroblasts, producing fibrils and even collagen if the deficiency be not complete. The proliferative zone of cartilage ceases to grow and is slowly resorbed becoming slender up to its end where it suddenly broadens into a thin junctional zone which is usually concave.

Adjoining this concave atrophic cartilage, in a developed scorbutic lesion, is a zone of fibrous tissue in which lie irregular, calcified and acellular fragments of the pre-existing trabeculae. This is the zone of the "Trümmerfeld" and "Gerustmark," long associated with scurvy and responsible for the characteristic "lattice" in radiographs. In our experience the amount of fibrous tissue formed at the costo-chondral junctions is roughly

proportional to the stresses present and is usually more pronounced in the lower than the upper ribs. If bone and cartilage are separated by strains the fibrous tissue response is magnified and forms a defective callus. These features indicate that the fibrous tissue is a defective response to normal stresses.

Associated with such fullblown lesions are hemorrhages and irregular masses of acidophilic material which Aschoff and Koch considered to be formed of fibrin. Schoedle, who appears to have been the first to describe this material, thought it was bone. This was also Höjer's view. Höjer observed the sequences leading to its canalization, demonstrated calcium in it both tinctorially and by ground sections. The material has two rather characteristic features, it lacks collagen and usually stains a brilliant red. Hart and Lessing, in their study of scurvy in monkeys, were also aware of this material and at first believed it to be fibrin. This view they surrendered when they were unable to find evidence of blood pigment, which should have been associated.

These unique and specific characteristics are almost immediately altered by the administration of vitamin C. The fibroblasts are promptly surrounded by a thin shell of osteoid material and resume their rounded form. Trabeculae rapidly form, irregularly at first and gradually becoming more orthodox in appearance until nothing remains to indicate that scurvy has been present.

The sequences in the periosteum are essentially the same. If the fibrous layer is pulled free from the bone excessive fibrous tissue is formed. This is of an inferior quality although the cells themselves appear to be normal. Bone formation ceases and is replaced by connective tissue. The response to vitamin C is as rapid as that seen within the medulla.

Mouriquand, who has contributed many interesting observations to the literature of scurvy, and Dauvergne describe a special form of periostitis in guinea pigs which was produced by a careful adjustment of the vitamin C intake to a level which caused hemorrhages to appear only after 30 days of feeding. This spontaneously disappeared and 3 or 4 months later chronic changes occurred in the skeletal tissue. These consisted of a productive periostitis, ankylosis and stiffening of the hind legs. Pseudoparaplegia sometimes resulted as well as muscular contractions and signs of generalized rarefaction of the bones. The condition has many features seen in cases of chronic arthritis and "rheumatism" in man and is interesting in view of the frequently expressed opinion that some of these cases, especially those developing in the winter and spring, are due to a partial vitamin C starvation. The disease in guinea pigs was found to be irreversible. Other causal factors may have been present. Some of the

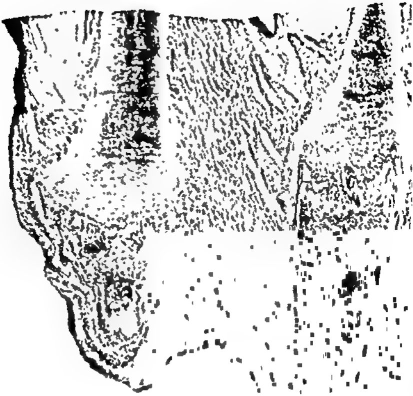
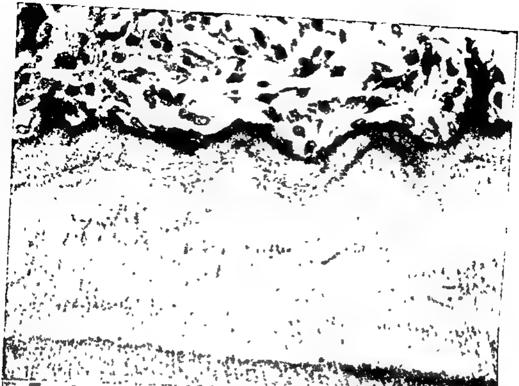


PLATE XXXIII Late skeletal lesions in experimental scurvy. Two c
junctions showing the characteristic appearance of scurvy The cart
and atrophic as well as the cortical bone Between the two lies a zone w
distinguished from the marrow by its lighter color It is composed c
tissue poor in collagen in which lie fragments of bone trabeculae Os
absent Note also the reaction outside the periosteum This is the le
responsible for the characteristic picture in radiographs.

Fig 1



2

PLATE XXXVI. The effect of complete deficiency of vitamin C for three weeks. Photographs of the teeth of guinea pigs Figure 1 shows apparent disappearance of the odontoblasts A zone of inferior dentin lies between pulp and preexisting dentin It contains no canals. The older dentin is rarefied, Tomes canals widened. Figure 2 shows an area in which odontoblasts may still be recognized as such although they fade into pulp structure.

lesions had an inflammatory character and may have been the result of the activation of a latent infection by faulty nutrition.

Histologic evidence of defective collagen formation occurs not only in the bones and ligaments but elsewhere. It occurs universally (Hojer). In the earliest stages it is abnormal, irregular and uneven. Later its formation is completely arrested. In surgical wounds three things are evident. The vessel endothelium proliferates but no new vessels are formed; fibroblastic activity is normal; no collagen forms. Wounds are therefore avascular and deficient in collagen. In the guinea pig skin, incisions do not heal (Wolbach and Howe). This is a typical and characteristic expression of the disease.

The Lesions in the Teeth

The incisor teeth of a guinea pig are a more delicate criterion of the presence of scurvy than the bones. Within four or five days the odontoblasts may be seen to shorten and become separated from the dentine by a faintly staining fluid zone. If the deprivation is complete, and is maintained until the death of the animal, usually about three weeks, they revert to a spindle form and are indistinguishable from the connective tissue cells in the pulp of the tooth. Simultaneously the Tomes canals widen appreciably producing porosis of the dentine and the teeth cease to grow.

If the deficiency is partial and prolonged for several months the odontoblasts produce a substance resembling bone which gradually fills the pulp canal. Finally, as the incisor continues to grow slowly the tooth becomes more and more completely replaced by this bone-like material (osteodentine) which is then surrounded by a thin shell of pre-existing dentine. If ascorbic acid be then given the cells which lie within the osteoid matrix promptly elongate and enlarge and revert to their original appearance and function.

The tooth pulp becomes hyperemic, the vessels dilated, thin walled and engorged with blood and the pulp tissue atrophic. The dental lesions commence in the crown and extend rootward. They are so uniform that before the development of chemical methods of assay they could be satisfactorily used for the quantitative estimation of the degree of deficiency present and therefore the antiscorbutic value of the diet. This was possible either by judging them by their histologic structure, the Hojer score, or by observing their rate of growth (Dalldorf and Zall). The normal rate of growth of guinea pig incisors is 0.7 to 0.8 mm. per day. In complete vitamin C deficiency, after a short period of lag, growth ceases. On partially deficient diets the rate of growth is roughly proportional to the amount of vitamin C in the diet.

Boyle, Bessey and Howe measured the rate of formation of denti[illegible] periodically injecting alizarin. They found a quantitative relation between the ascorbic acid administered and the thickness of the d[illegible] formed during the period of administration.

Harman and Kramer describe irregular, round elevations on the infer[illegible] surfaces of the mandibles in scorbutic guinea pigs. The bone was spon[illegible] in character and easily pierced with a needle. We have observed the lesions which are quite regularly present in protracted scurvy. The[illegible] appear to be the consequence of rarefaction plus stress. The teeth ar[illegible] pressed into the mandible which is unable to resist and bulges into the periosteum. Höjer described these lesions in detail. They constitute a further example of the relation of stress to the lesions of scurvy.

Fish and Harris have studied the teeth of scorbutic guinea pigs and describe enamel defects which they believed might be related to caries. Their report is illustrated with several excellent longitudinal sections of incisors, both normal and scorbutic. Boyle has pointed out the defect in their observations, namely, that the degree of scurvy was sufficient to weaken the peridontal tissues and lead to dislocation and separation of the tooth and enamel organ. This explanation not only agrees with other known facts about the disease but is supported by Boyle's own results. As is shown in the discussion of avitaminosis A enamel defects and lesions of the enamel organ itself are the consequence of that deficiency. Indeed the enamel is disproportionally thick in scurvy due to atrophy of the dentine.

Boyle has described the effects of scurvy on the alveolar bone and states that vitamin C deficiency is the only nutritional disease, in his experience, which produces the characteristic features of systemic pyorrhea. A necessary distinction must be made between the local pyorrhea which develops about impacted food or from disuse. In cases of generalized pyorrhea a relative immunity to caries is quite common. After several months of a partial vitamin C deficient diet Boyle's animals showed peridontal weakness and many teeth, but particularly the molars, became displaced. Boyle states that all of the patients of a large dental clinic whose blood plasma ascorbic acid was less than 0.5 mgm. per 100 cc. have shown similar gingival lesions which respond to specific therapy.

Lesions in the Vessels

By analogy it is believed that a similar inferior intercellular material is responsible for the hemorrhagic diathesis present in both human and experimental scurvy. Unfortunately no histologic evidence of such a change may be seen in the capillaries, but Wolbach has thoroughly demonstrated that while there is no lack of growth capacity in the capillary endothelial

Fig. 1

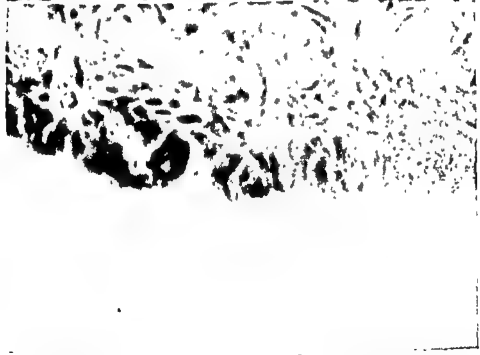


Fig. 2

Plate XXXVII Experimental scurvy. Effects of recovery on the teeth of guinea pigs. Figure 1 shows the reappearance of odontoblasts and the formation, adjacent to them, of a zone of new dentin. Figure 2 shows more advanced repair. These photographs are of animals depleted as those in Plate XXXVI and then given vitamin. (*Reprinted by permission of the Journal of Experimental Medicine.*)

Fig 1

Fig 2

PLATE XXXVIII Experimental scurvy. Lesions in the skeletal muscles Figure 1 shows the early effects of deficiency in which fragmentation of the muscle fibers is associated with intense activity of the sarcolemma The attempt at regeneration does not progress far and the late results (fig 2) show loss of muscle fibers and replacement by collagen-poor connective tissue. Hyalinized fragments of muscle persist Such lesions are seen in other deficiency disease but the repair is rather characteristic of scurvy.

cells fully formed capillaries do not occur during complete deprivation. Furthermore the demonstration that the capillaries lose their fragility within a few hours of administering vitamin C suggests that something comparable to the results of treatment of the dental lesions also occurs in the vessels. Müller suggests the principle alteration is in the supporting mesenchyme of the smaller vessels and capillaries.

In the older literature mention is made of various vascular lesions in scurvy, fatty and hyalin degeneration and inflammatory processes which were considered responsible for the vascular weakness. Aschoff and Koch were unable to substantiate these results in their material and such lesions do not occur in experimental scurvy.

All of the manifestations of scurvy are greatly modified by the presence or absence of stress, and all of the lesions may be understood if this factor and the basic one of defective intercellular substances are appreciated. Stress determines the location of the hemorrhages. Protected vessels rarely rupture; stress augments the Gerüstmark and constitutes an essential element in the production of the muscle lesion (vide infra).

The hematopoetic tissue in the bone marrow is not characteristic in scurvy. The capillaries and small veins are greatly dilated during the early stages of the disease and somethimes these dilated vessels simulate small hemorrhages. Hemorrhages occur also but these are usually near the epiphysis. Höjer agreed with Moore and Jackson that the larger vessels had inordinately thin walls. This was thought the result of atrophy of the collagen. Hess' view that the hyperemic marrow of rickets contrasts with the paucity of vessels in scurvy appears to be true only of the late stages of scurvy. In the early periods the marrow is hyperemic. Schmorl's theory of the pathogenesis of scurvy was that it was a direct result of the anemia of the marrow. At that period vessel lesions were considered the fundamental disturbance in scurvy.

Lesions occur in the muscles in scurvy. Höjer felt that the myopathy had characteristic features, among which were pronounced necrosis with a tendency to calcification and the formation of giant cells which represent abortive attempts at regeneration. These cells have been generally interpreted as multiplying sarcolemma and their rate of growth may sometimes be so excessive that they suggest a myosarcoma. Excepting for the calcification, which has rarely occurred in our own material, the lesions are indistinguishable from those produced by other dietary deficiencies (Pappenheimer) or by other means (Forbus). Höjer ascribed the weakness which appears early in scurvy to the myopathy and described similar changes in the heart muscle to account for the cardiac weakness. He found myocardial lesions in ten of thirteen animals but the most extensive

lesions were in animals infected with tuberculosis. We have never seen cardiac lesions comparable to those described by Höjer. Fatty degeneration of the myocardium is common.

Scurvy produces atrophy and degeneration of the germinal epithelium as Medes has shown, and early destroys the ability to sire litters. In the female the oestrus rhythm is maintained in moderate deficiency but is suspended if the deficiency is severe enough to produce emaciation. The adrenals atrophy. This may be due to loss of cortical fat which is reduced

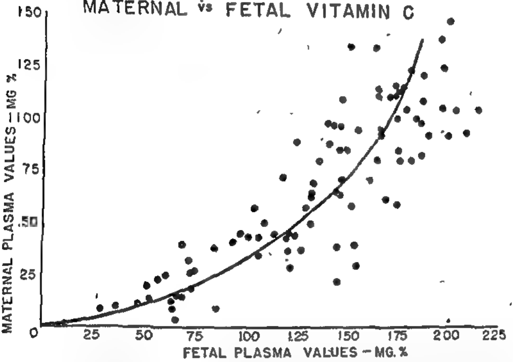


FIG 38 Relationship between maternal and fetal vitamin C plasma concentrations. In each case the maternal value has been plotted against that of her infant. The fetal values are higher than the maternal but the ratio varies greatly depending on the level of the maternal concentration. (Courtesy of Dr. Curtis J. Lund)

in the terminal stages of scurvy. The reduction in cortical fat is related to the loss of vitamin C in the same tissue as Bessey, Menten and King demonstrated with the silver nitrate reaction. Atrophy of the lymphoid tissue of the lymph nodes and spleen is pronounced and some atrophy occurs in the salivary glands, kidneys, and liver. Hemorrhages and erosions of the stomach and intestinal tract have been reported by several investigators. They were noted by Holst and Frolich in the duodenum. More recently Smith and McConkey reported an incidence of approximately 25 per cent most of which were in the first part of the duodenum. These

lesions were erosions, usually covered with a small blood clot. Similar lesions have been reported in human cases.

Scorbutic guinea pigs frequently have premature litters. The young show retarded growth and many are dead. In cases of partial deficiency the young may apparently be born partially depleted of vitamin and quickly become scorbutic themselves unless the maternal milk is supplemented with vitamin C (Ingier). The pregnant animal is relatively immune to scurvy. Symptoms develop rapidly after parturition.

A moderate secondary anemia occurs in experimental scurvy and promptly responds to vitamin C. The blood changes have been studied by Meyer and McCormick. Höjer believed that the regeneration of both erythrocytes and granular leucocytes was retarded but that anemia and leucopenia were dependent on excessive destruction of the cells in which case the faulty hematopoiesis became apparent. Leucocytosis failed to develop in his scorbutic pigs when they were infected. The bone marrow is natural in appearance. Presnall found the coagulation time increased 54 per cent and the platelets reduced 36 per cent. The decrease in platelets is paralleled by the decrease in erythrocytes Israels examined the bone marrow in three cases of scurvy both before and after treatment and reports diminished erythropoesis which responded promptly to treatment. This is a nonspecific effect shared by many food factors.

Mention is frequently made of edema in experimental scurvy. Moore and Jackson, whose work was very important in inaugurating the more recent study of the disease in guinea pigs, spoke of the edema about the joints and bones. The basal diets used were not complete. Doi has recently demonstrated diminished plasma proteins and increased water content in scurvy which appears to have been due to the deficiency itself. Hojer's view, expressed before Doi's study, was that edema was due to an associated deficiency. On a complete basal diet his animals were free of edema. Some of the fluid about the bones in scurvy may represent unjelled intercellular material.

The scorbutic guinea pig characteristically sits in a hunched position with drooping head, the so-called "tooth-ache position," has swollen and tender joints, and, if the disease is severe, is greatly emaciated. The guinea pig requirements of vitamin C are relatively large. According to Dann and Cowgill approximately 0.5 mgm. ascorbic acid is needed for each 100 grams body weight. However considerably larger amounts of antiscorbutic vitamin are required to prevent all of the manifestations of deficiency. Three mgm. of ascorbic acid are needed to insure natural tooth growth and nearly as much to prevent histologic lesions in the incisor teeth in pigs of 250-gram size. In the guinea pig as well as man there therefore

appears to be a degree of vitamin C underfeeding in which distinct morbid effects are present without the clinical or grosser manifestations of scurvy.

Abercrombie described lesions in the thyroid gland in guinea pig scurvy. The follicles were irregular, their cells columnar; colloid was reduced and the interfollicular cells increased.

Giroud has been most active in studying scorbutic tissues by means of silver stains which he believes selectively color the ascorbic acid within the cells. There is still some uncertainty as to whether the method is specific for ascorbic acid but there can be no doubt that many of the phenomena of scurvy can be followed by this technique. Giroud's monograph should be consulted. These studies and somewhat similar ones by Glick and Biskind have related the site of storage of ascorbic acid to definite portions and cells of the tissues. The glands of internal secretion have been most thoroughly studied since these contain the largest amounts of the vitamin and relationship to function would seem especially close. Thus the zona fasciculata of the adrenal has been shown to have double the vitamin content of the medulla. In the pituitary gland the pars intermedia is especially rich in vitamin. Fluctuation in the ascorbic acid content of luteum tissue has been correlated with its functional activity and parallels the concentration of progesterone.

Some mention is made later on of the nervous symptoms in human scurvy. Few observations have been made of the nervous system in the experimental disease. Meyer and McCormick reported peripheral degeneration and changes in the motor cells in the anterior horns. No functional disturbances have been observed.

In certain groups of guinea pigs fed the Sherman-LaMer diet fatty livers are common. This is not related directly to the degree of scurvy nor is it constant. Spellberg and Keeton ascribe it to the combined effect of ascorbic acid and an unidentified dietary factor. It has been repeatedly re-discovered during the past 20 years. Russell and Callway's attention was directed to the kidneys of their scorbutic guinea pigs because they were stained darkly when trypan blue was injected. They associated this phenomenon, for lesions were not present, with the abnormal catabolism of aromatic amino acids which Levine, Gordon and Marples reported in infants and corrected by ascorbic acid.

MORBID ANATOMY OF HUMAN SCURVY

The significant lesions in human cases of scurvy are the hemorrhages and the skeletal defects.

The hemorrhages may take place in any organ and may vary from petechial size to huge extravasations. On the skin they are located about the hair follicles, sweat glands and skin lesions. They are common over

bony prominences and in infants on the inner surfaces of the thighs where the diaper rubs. The deeper hemorrhages follow the fascial planes. Massive hemorrhages occasionally cause death in scurvy as in a recent case where a huge hemorrhage occurred within the pericardium.

The skeletal lesions are identical in their minute structure with those in the guinea pig and the commonest sites of the lesions are the costo-chondral junctions, the distal ends of the femurs, proximal ends of the tibias and femurs and the wrists. The distal end of the humerus and the proximal end of the ulna are usually spared. A feature of the bony lesions in man is the conical widening of the ends of the long bones. In severe cases chest deformities are seen which have been described as a bayonet deformity when the dislocated diaphysis pushed deeply into the Gerustmark.

The muscle lesions are identical with those which occur in guinea pigs. They presumably occur only in severe cases. Hojer's view that they were responsible for the early weakness in scurvy is not supported by anatomical evidence that they actually occur at that time. The most extensive description of them in human material may be found in Mahé's monograph. Mahé pointed out the striking relationship to stress. Soldiers develop muscle lesions in the thighs and buttocks, artisans who work with arms and hands develop muscle lesions there, etc. The lesions are usually complicated by hemorrhages.

Lesions also occur in the teeth of man. Westin described eighteen cases, some after recovery and others with various degrees of scurvy. The lesions in the pulp include hyperemia and edema with atrophy, degeneration of the odontoblast layer with the formation of small cysts, destruction and calcification of the vessels, and necrosis and calcification of areas in the pulp. Osteoid tissue forms about the calcific masses and calcified vessels. The dentine undergoes porotic changes, with resorption along Tomes' canals which widen into spindle and round spaces. The formation of abnormal and irregularly canalized replacement dentine occurs. Similar changes occur in the cementum. Lesions are most common in the apical third of the tooth and at the bifurcation of the root canal. In acute cases fluid was seen about the odontoblasts.

Westin also demonstrated lesions in the teeth before the development of bone lesions. The parallelism between experimental and human scurvy is, therefore, complete in all studied features. Since Walkhoff's demonstration of congenital scurvy in the guinea pig with lesions of the teeth, we may anticipate that congenital dental lesions in man will some day be described.

Conspicuous changes occur in the mouth. The gums become swollen and bleed easily, and rarefaction of the alveolar bone causes the teeth to loosen and fall out. The characteristics of the gingival lesions are a rapidly

developing hyperplasia of the papillae with a tendency to spontaneous and intractable hemorrhage, disintegration of the epithelium, commencing on the papillae and followed by the development of granulation tissue and finally gangrene (Westin). Trauma obviously plays a large part in localizing these lesions since they do not occur at all unless teeth are present, and then at sites of greatest irritation and stress. Scorbutic lesions of the mouth are strictly limited to the gingiva in contrast to the mouth lesions of various factors of the vitamin B complex.

The skin lesions are petechiae which occur, characteristically, about the hair follicles. This is probably due to the rich plexus of small vessels there and the minute traumata they are exposed to by contact with the unyielding follicle. The keratotic changes so often associated have been present in certain epidemics and not in others. In Aschoff and Koch's cases, drawn from various nationalities in the Balkans the incidence was high among certain races and not in others. This we believe to have been due to differences in eating habits. Vitamin C deficiency must have been widespread but the diets varied in vitamin A values depending on the eating and cooking practices of the different nationalities. Two factors make it extremely doubtful that the lesions are scorbutic. They may or may not occur and they are by structure and incidence typical expressions of vitamin A deficiency.

Patients with scurvy are commonly pale and anemic looking, and frequently show slight edema about the ankles. In the older literature anasarca was frequently described. Hydropericardium and hydrothorax are often noted. The heart is frequently enlarged. Erdheim found enlargement in twenty-one of thirty-one cases. The enlargement of the heart is due to hypertrophy of the left ventricle. The stomach is frequently congested or stippled with petechiae, and small duodenal ulcers are not uncommon.

Infantile scurvy is rare before the fifth month of life. Hill has described a case in a Chinese infant three weeks old. Jackson and Park have reported a typical case of scurvy in an infant twenty days old. Symptoms had been present from birth. The diagnosis was confirmed by autopsy.

Scurvy and Senility

Aschoff and Koch were greatly impressed with the similarity of the scorbutic lesions in the skeleton to those in senility. The changes in cortical bone are difficult, if not impossible to distinguish. The distribution of the lesions is not the same in the two conditions but this may be due to differences in mode of life. In both conditions the bones are notably thin and rarefied, susceptible to fracture and defective in the ability to form callus once fracture has occurred. The suggestion seems a worthy one

and would be included in the discussion of the significance of vitamin C in modern life were it not for the problem of pathogenesis it raises.

Westin interpreted the tooth lesions as similar to the atrophy of old age and said scurvy may be considered to hasten involution. In his cases the teeth showed the same resistance to caries that is seen in senility as well as the rarefaction common to advanced years. Fish and Harris pointed out that the osteodentin formed in scurvy is "normally" present in senile teeth and represents a scar about dentin which has died. Höjer considered the primary disturbance in scurvy to be atrophy of skeletal parenchymal cells, in the case of the teeth, the odontoblasts.

It is extremely doubtful that the problem can be resolved by histologic study alone. A comparison of the irregular dentin, or osteodentin produced in human teeth about carious cavities in well fed and scorbutic subjects might clarify the subject. The experimental studies seem to us to be much more significant because of the uniformity of the subjects and the ready availability of all stages of the lesions. Wolbach and Howe's observations in complete scurvy are substantial evidence. It is difficult to correlate such a phenomenon with the decadence of senility. We are familiar with no other adequate explanation of their results than that, in the absence of vitamin C, the odontoblasts form an inferior, substitution material. That similar material (osteodentin) forms in man in a variety of conditions does not establish that in the case of scurvy it is not defective in quality. Indeed the extent of its formation in human teeth, in scurvy, suggests that it may be inferior and that, being so, larger amounts are required to afford the support and defense the rarefied tooth requires. "The decline in vitamin C content of human tissues after the age of 45 indicates an increased rate of destruction or wastage of this vitamin in older people" (Sherman). It is also known that larger amounts of ascorbic acid are required to saturate elderly people.

Possibly the rôle of vitamin C in the control of tissue respiration is the clue which will aid us in our understanding of senility.

The Anatomical Diagnosis of Scurvy

The anatomical diagnosis of scurvy can be made from a gross examination of the tissues if the case has progressed to an advanced stage. In other cases the occurrence of unexplained hemorrhages or muscle degeneration requires the histologic examination of samples of the skeleton. In the costochondral junctions various stages of the typical scorbutic lesion will frequently be found. It is not necessary that it be fully developed. Experience rapidly recognizes a general fragility of the connective tissue fibers, a suggestive watery zone about the osteoblasts and a defective osteoid tissue formation which plainly indicate the presence of scurvy.

It is often difficult to distinguish scurvy from other dystrophies of bone such as osteogenesis imperfecta. The distribution of the lesions and the presence of small hemorrhages are helpful in these cases. If suspicion exists at time of necropsy specimens of the liver and adrenals should be taken for measurement of the ascorbic acid content.

THE DIAGNOSIS OF SCURVY

The clinical recognition of scurvy is relatively simple if the morbid anatomy is appreciated. The cardinal symptoms are a hemorrhagic diathesis, weakness and signs and symptoms related to skeletal lesions and hemorrhagic gingivitis. In the present chapter the commonest symptoms will be described as well as the manifestations of partial ascorbic acid deficiency for while florid scurvy is at present a medical rarity mild forms of the disease are quite common.

Incubation Period

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In the second case Dr. Schick lived under similar conditions for 160 days. Depletion, as measured by the plasma values, developed at the same rate. The blood value remained at a level of 0.05 mgm. from the 116th to the 154th day. No symptoms were noted although the gingival papillae were somewhat reddened and eroded but this was said to have been true before the experiment started. van Eekelen lived on a vitamin C free diet for 84 days without noticeable effects but Widenbauer, who fed a deficient diet to two idiot infants reported tenderness of the thighs and radiographic evidence of scurvy after 3 months. It therefore seems evident that the incubation period of scurvy can be a matter of months if the individual

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Clinical diagnosis of milder cases of scurvy is difficult and uncertain. Frölich has reported eight characteristics which he considers helpful. These are dystrophy, anorexia, anemia, occasional slight edema, cessation in gain of weight or loss of weight, susceptibility to infection, intestinal disturbances and now and then more pronounced symptoms such as hematuria. He considers that the diagnosis is never definite in such cases until the effect of the administration of the vitamin is noted.

Adult patients suffering from scurvy complain of weakness, pains in their legs, swollen, bloody gums and hemorrhages. Examination discloses petechiae, chiefly about the hair follicles of the lower extremities and sometimes brawny, tender thighs. All of these features are due to hemorrhage and it is interesting to recall that older writers considered a hemorrhagic diathesis as pathognomonic of scurvy. Recent work shows that this opinion is more justified than was thought a few years ago. The hemorrhagic diathesis is the essential manifestation of vitamin C deficiency, is responsible for most of the signs and symptoms of the disease and a diagnosis of scurvy cannot be made in the absence of a tendency to bleed.

Weakness is usually the first thing complained of by persons suffering from vitamin C depletion. Fatigue, palpitation and breathlessness are also common. The patients dislike to stand or walk and often affect a rather characteristic standing position with their legs slightly flexed. The complexion is pallid and dirty looking. Gingivitis occurs, followed by loosening of the teeth, a consequence of resorption of the alveolar bones and infections abut the teeth and is accompanied by a foul breath. Other signs of scurvy are hematuria, bloody diarrhea, nasal hemorrhage or hematomas about the jaw or bones of the lower extremities.

The hemorrhages were considered as pathognomonic of the disease by older writers. They may occur in any organ and may cause confusing diagnoses. Thus hemorrhages in the lower right quadrant have been mistaken for appendicitis and hemorrhage in the transverse colon for a neoplasm. A diffuse, firm and infiltrating hemorrhage in the thigh was first considered to be a sarcoma. A pericardial hemorrhage caused death in a case described by Barton and Freeman.

Fever is usually present at some period of the illness and complicating infections are extremely common. If death is not due to intercurrent disease it comes suddenly with syncope.

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The fever which often occurs during the course of both experimental and human scurvy has never been satisfactorily explained. Mouriquand, Pouzet, Schoen and Belly have recently described a case of scurvy in a girl two and one-half years old in which the fever was constant until orange juice was given. It then sank to normal only to reappear when the antiscorbutic was reduced. The original dose of three lemons and four oranges was resumed and the temperature became permanently normal.

Many writers have divided scurvy into different forms or stages. In animals, where the diet is rigidly controlled, this can be successfully done, as witness Höjer's classification. In human cases a natural division seems possible between manifest and latent scurvy, to which has recently been added subclinical scurvy. Older classifications of manifest scurvy seem of interest chiefly because they demonstrate that the severity of the case is expressed by the extent of the hemorrhagic diathesis. In the first phase of scurvy, according to French writers, slight bleeding of the gums and small hemorrhages about the hair follicles ("Piquete scorbutique") are found. In the second phase ecchymoses, especially about the lower leg and knee, are conspicuous. In the third phase extensive hemorrhages from the mouth, nose, stomach and bowel and extensive skeletal hematomas develop. The tendency to bleed is therefore not only the salient clinical feature of scurvy but varies in degree with the severity of the particular case. In the mildest forms, the subclinical cases, a weakness of the smaller vessels exists without spontaneous rupture and can be demonstrated by measuring the capillary resistance to artificial stresses (the capillary resistance test). Aschoff and Koch spoke of the vascular changes as "angiodystrophy."

There are no essential differences between scurvy in infancy and adult life and in both cases varied manifestations of the tendency to bleed easily, pallor and rheumatic pains are the salient features. The confusion that formerly led to a separation of infantile scurvy (Moeller-Barlow disease) from the disease in adults was due to a combination of rickets and scurvy, often with other dietary deficiencies as well. E. Fraenkel's case of a seven year old boy with hemorrhages about the joints and skeletal lesions of rickets illustrates the difficulties of older writers. In adults, of

PLATE XL. Scorbutic gingivitis Swollen, boggy, hemorrhagic papillae with ulceration about the middle incisors.

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course, scurvy is rarely complicated by lesions of the skeletal system other than those characteristic of the scurvy itself. The diagnosis must frequently be made between scurvy and rheumatism and between scurvy and blood dyscrasias. A satisfactory hematological examination usually suffices in the latter instance and the therapeutic test is of great assistance, especially since the isolation of the vitamin which has made the parenteral administration of large doses feasible.

A moderate degree of anemia is usually present in scurvy. This is of a microcytic or normocytic type and is distinguishable from other secondary anemias only by therapeutic test. Reticulocytosis and recovery follow the use of vitamin C while iron and liver extracts are ineffectual.

Optic proptosis is a late symptom of scurvy. In 1898 the American Pediatric Society reviewed 379 cases of scurvy. In 40 proptosis had occurred. The lesion appears suddenly as a result of hemorrhage either beneath the periosteum or into the orbital fat. The blood pigment sometimes moves forward to form a blue line along the lower orbital margin.

"Scurvy Sclerosis" is the term given the tender, brawny hematomata which may occur in the thigh in scurvy. We have seen this lesion in elderly patients on two occassions.

Of the cutaneous manifestations of scurvy, other than the perifollicular petechiae, are the keratosis suprafollicularis present in certain of Aschoff and Koch's cases, the scorbutic pemphigus of Lutton and the scleroderma like lesions described by Morawitz and Pfeiffer. Of these the most frequently mentioned is the keratosis suprafollicularis and this is almost certainly the result of a simultaneous vitamin A deficiency. Thus Mahé describes lesions he designates as lichen pilaris which are identical with those due to vitamin A deficiency; hard, elevated follicles with huge extruding masses of concretions giving the skin a pebbled appearance. The hairs were often broken off and stood like bristles or were lost entirely. Lind said the skin was usually soft but sometimes quite rough "like the skin of a snake." The epidemics observed by these early authorities were associated with other signs of vitamin A deficiency. Nightblindness was very common in Krebel's cases, so commonplace that its occurrence in scorbutics attracted little attention. Some early writers considered nightblindness a predisposing cause of scurvy and Hulme, writing of the epidemic of scurvy in the English fleet in 1761 spoke of nightblindness as the most important symptom. Under these circumstances vitamin A deficiency dermatosis could be expected to be present and the records show it was. The best contrary evidence is the development of similar lesions in a human volunteer maintained on a diet deficient in ascorbic acid but containing large amounts of vitamin A and shown to be free of signs of vitamin A deficiency (Crandon and Lund).

Ecker and Pillemer followed the complement titer in two classical cases

of scurvy. Before treatment the titers of complement were very low, as was the blood ascorbic acid. Treatment caused a parallel rise in both until the blood ascorbic acid passed the 1 mgm. per 100 cc. value after which no further increase in complement titer occurred. This corresponds to the blood level associated with maximum complement activity in guinea pig scurvy and the authors suggest that complement is a good biological index to scurvy. Very similar results have been reported by Chu and Chow who studied, in addition, cases of subclinical scurvy and found a correlation between blood ascorbic acid and complement in these cases also.

More recently hemolytic complement has been carefully followed in monkeys and guinea pigs by Chapman and Harris who found no diminution in complement throughout depletion or during severe and even fatal scurvy. The administration of ascorbic acid was likewise without influence. Whether this discrepancy is due to the methods used or differences in the basal ration is uncertain. It appears extremely doubtful from the observations of Chapman and Harris that a relationship between complement and vitamin C actually exists.

Subclinical Scurvy

Mild, atypical cases of scurvy are much more frequent than clinically definite ones. They masquerade as rheumatism, gingivitis, purpura, hemophilia and osteomyelitis. They may be definitely diagnosed only by demonstrating depletion of ascorbic acid and cure by specific treatment. There usually is little question about the diagnosis if scurvy is considered.

But there are still more individuals who lack even these symptoms of scurvy and yet who prove to be depleted of vitamin. It is a natural feature of the avitaminoses, in contrast to the major infectious diseases, for example, that all degrees of deficiency, and therefore presumably of ill health may occur. For these conditions the term "subclinical" scurvy has been proposed.

This conception rests on rather substantial ground. Scurvy has been long recognized to have an asymptomatic stage which precedes the characteristic symptoms and, which is more important, a degree of scurvy is recognized which is without definite symptoms although well developed and characteristic lesions may be present. The experience of Park and his associates is especially illuminating. In reporting 125 cases of scurvy in infants Park emphasizes the circumstances which made possible the recognition of most of these cases. Twenty years previously Dr. Martha Elliott inaugurated thorough radiographic and anatomic study of children dying from any cause, principally to determine the incidence and character of rickets. Out of this material, comprising 532 cases, 125 instances of scurvy appeared. In many cases diagnosis rested exclusively on histologic examination or on histologic and radiographic evidence. It was found

that both of these methods might yield definite evidence of scurvy in the absence of symptoms of a recognizable kind and it was further discovered that even the anatomic diagnosis of the disease was frequently missed unless the pathologist was very alert to it. In other words the diagnosis of scurvy is difficult and frequently missed and the disease may exist in a well developed form without symptomatic clues to its presence. The full significance of this is not yet understood but modern studies have yielded engrossing results concerning the prevalence of such cases. The same is true of experimental scurvy. Eddy noted that lesions could be demonstrated in animals fed sufficient ascorbic acid to prevent symptoms and Dalldorf and Zall demonstrated that to insure normal growth of guinea pig incisors a much higher level of vitamin was required than was needed to prevent lesions. Zilva found that 10 times as much ascorbic acid was required to saturate a guinea pig than to prevent clinical scurvy. There is, indeed, complete agreement that such a condition of depletion exists and can be demonstrated at will. The issue is whether it is important.

Zilva concluded that it was not, that saturation did not represent optimal conditions. Szent-Györgyi on the other hand states that clinical signs are late, premortal evidence of deficiency, that the subclinical state predisposes to infection and other diseases and has a great significance. The seemingly tremendous amount of vitamin necessary to saturate a guinea pig is not more than an animal living naturally in the tropics would consume. It is not fair to base requirements on the behavior of a caged animal. This is much the point of view taken by Giroud who believes a fair normal requirement would be the amount necessary to maintain organ concentration at those levels which non-susceptible animals have. Thus the rat adrenal quite constantly contains 1 mgm. or more ascorbic acid per gram tissue while the value in the guinea pig fluctuates greatly. Since the adrenal concentration of an animal independent of dietary supply is 1 mgm., says Giroud, we may assume that this is the amount necessary for the proper function of that particular tissue.

It may be seen, therefore, that there are at least two conceptions of what is meant by subclinical scurvy, an asymptomatic form of deficiency and a mild, atypical form of symptomatic scurvy. To which might be added a third type; cases of vitamin C depletion or atypical scurvy associated with or due to other diseases. A good example of the latter is the situation which exists in cases of chronic peptic ulcer.

Peptic Ulcer and Vitamin C

Schultzer studied patients with gastric ulcer who were placed on the usual therapeutic diets. One-third of the group had increased capillary fragility after sixteen days. In 70 per cent of these, treatment effected

a return to normal. Many authors have confirmed this. Croft and Snorf found 15 of 18 patients with peptic ulcer had blood plasma ascorbic acid values below 0.4 mgm. per cent. Harris, Abbasy and Yudkin found the average excretion of such patients to be only 5.6 mgm. per day (approximately one-third normal). Portnoy and Wilkinson studied large groups of normal controls, miscellaneous ward controls, patients with peptic ulcer and patients with hematemesis by various methods. The urinary excretion of the groups averaged, in order, 29, 17, 7 and 7 mgm. per day. The saturation test showed that from 2 to [illegible] grams of ascorbic acid were required by the last 2 groups. This considerable variation probably is due to the same factor which prevented 30 per cent of Schultzer's cases from responding to diet. The blood plasma of the peptic ulcer and hematemesis cases was at or very near the scorbutic level, 0.14 to 0.59 mgm. per cent. Nielsen had similar results. Lazarus found 10 of 12 cases of hematemesis unsaturated, 7 were extremely deficient. Ingalls and Warren examined 20 cases of peptic ulcer of which 18 were found to be depleted. The average blood plasma value was 0.19 mgm. per cent. Euler and Otto, Chamberlin and Perkin, and Bourne have all described similar results. Bourne used capillary tests as his criterion.

It would seem reasonable to conclude that patients with peptic ulcer are characteristically depleted of vitamin C. Yet there is little evidence that they have symptoms of scurvy, unless the associated bleeding is partly due to scurvy. Contrarily peptic ulcer is an uncommon complication of scurvy. Scorbutic animals frequently have small hemorrhages in the mucosa of the stomach but not ulcer. One is forced to conclude that either the dietary of ulcer patients is grossly deficient, their requirements abnormally high or their utilization imperfect. Do these low blood values have any significance in the handling of patients? Taffel and Harvey have measured the tensile strength of surgical wounds in guinea pigs on normal and deficient diets. The scorbutic animal has a stronger wound on the 4th post operative day but a weaker one on the 6th day. Even the partially depleted animals had demonstrably poor union on the 8th and 10th days, due in all likelihood to the defective collagen formation characteristic of scurvy. This possibility should be considered. Another application may prove to be the usefulness of vitamin C in the treatment of hematemesis. We have followed a considerable number of such cases and frequently observed hemorrhage cease after large doses of ascorbic acid. Replacement therapy might improve the status of these patients in other ways. Any efforts along such lines will require rather large amounts of ascorbic acid.

In like manner many cases of hemorrhage may be related to a stage of vitamin C delpletion. Epistaxis is certainly one of the most common.

We have followed a number of cases in which recurrent epistaxis, without obvious organic cause, was found associated with ascorbic acid depletion and responded immediately to specific, replacement therapy. Selected cases of menorrhagia and metrorrhagia behave similarly. Frequently cases which seem at first examination to be purpuras or other hemorrhagic diseases may be explained on a vitamin deficiency basis. But can we apply our knowledge of scurvy to still more unrelated conditions?

One of the lesions of scurvy is degeneration and fragmentation of the skeletal muscles, Zenker's degeneration. Study of older records shows how consistently it is present during scurvy. Mahé reviewed the older literature. The muscles turned to "currant jelly" and were sometimes so extensively diseased that the patient was unable to stand. The same degeneration occurs in experimental scurvy. Histologically it is identical with Zenker's degeneration as seen in typhoid fever. It would seem reasonable to inquire whether the degeneration in the muscles is due to typhoid fever per se or to a secondary form of vitamin C deficiency. The evidence is only suggestive but illustrates what may well be a profitable point of view. Thus 20 years ago typhoid fever was shown to produce a negative nitrogen balance. We now know that, in common with most other febrile diseases, the ascorbic acid balance is also negative. The late stages of typhoid fever are complicated by hemorrhage. The intestinal bleeding is generally ascribed to the ulcers but oozing is also very common. Epistaxis is known to be a relatively common complication. Occurring in the late stages of the disease it often warns of impending death. Thus typhoid patients may be assumed to have depleted stores of vitamin C, inadequate intake (certainly in the days in which Zenker's degeneration was common), an unexplained hemorrhagic diathesis and degeneration of their muscles. To which might be added Beneke's interesting observation that all of the infants he examined post mortem during the war which died of sepsis had Zenker's degeneration. Since that time it has become relatively uncommon. The War period in Germany is known to have been a period of endemic subclinical scurvy as the papers of Meyer and Nassau show.

Other causes, including nutritional ones, are known to produce Zenker's degeneration. But the most likely to complicate typhoid fever seems to be scurvy. The response of typhoid patients to high calorie diets was proven by Coleman twenty years ago. Could not more be done for these people now if the balance of vitamins as well as nitrogen were considered? It is an interesting possibility.

Special Tests for Ascorbic Acid Deficiency

We are surfeited with tests for ascorbic acid deficiency but still hungry to know what deductions can safely be drawn from them. It is impossible

to compare all the procedures which have been described. The present discussion will be limited to those with which we have had personal experience. The latter fall into one of three groups; tests of capillary fragility, of the blood or urine concentration of ascorbic acid or of the degree of saturation. (Technical instructions may be found in the appendix.)

The measurement of capillary fragility antedates the chemical tests by many years. Auspitz (1881) drew conclusions from cupping patients with scurvy which seem to be the earliest references to this phenomenon. Hess performed many tests using a tourniquet to congest the vessels and determine whether they were fragile or not. Later Hecht devised a small glass cup connected with a variable negative pressure system and a manometer. Thus two methods exist for measuring the strength of the capillaries, one using positive and the other negative pressure. Both tests doubtless respond to the same conditions and essentially the same results have been secured with each. The reports of Wright et al. and Göthlin should be consulted for studies based on the tourniquet test.

What evidence exists that capillary fragility is a measure of scurvy? It is first of all generally accepted that the hemorrhagic diathesis is the primary or one of the primary, characteristics of the disease. But there are various other reasons.

1. Capillary fragility is present in most cases of manifest scurvy and its intensity is related to the degree of the scurvy.

2. It responds immediately to parenterally administered ascorbic acid and slowly to oral medication, as scurvy itself does (Dalldorf and Russell, Adant and others).

3. The seasonal fluctuation in capillary fragility parallels that of vitamin C intake and of serum complement (discussed elsewhere).

4. It is absent at birth when blood vitamin and stored vitamin are present in high concentrations and appears during the first year of life when the infant's ascorbic acid supply falls (Hoffman).

5. Capillary fragility is more common in bottle than breast fed infants. Children with vitamin C supplements have more resistant capillaries than comparable children without supplements (Roberts, Blair and Bailey). Fragility is a common characteristic of poorly fed children (Dalldorf).

6. Capillary fragility is present in infants with idiopathic hemorrhage, intestinal bleeding (Ratnoff), pachymeningitis hemorrhagica interna and erythrocyturia minima (Hoffman). It has been prevalent to a pronounced degree when latent scurvy has been prevalent (Meyer).

7. Many of the conditions associated with capillary fragility which were formerly not believed related to scurvy are now known to be associated with vitamin C depletion. Among these are bottle feeding, infectious diseases (in particular, whooping cough, scarlet fever), and thyroid medication.

The weakness of the test is that certain other conditions which evidently are not related to scurvy also cause fragility. Thus Elliott's studies of thrombocytopenic purpura show that fragility is closely related to the course of that disease. Weld has demonstrated that ultraviolet irradiation alters the capillary resistance. Changes also occur from chemical and biological toxins, diurnally and between different parts of the body and

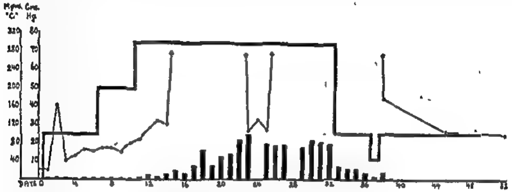

FIG. 39. Effect of ascorbic acid on capillary resistance and urine content of vitamin C in a severe case of scurvy. *Mgs. 'C'* represents the values of vitamin. The heavy line indicates the intake, the total excretion during the six hours following administration is shown by the solid columns. All of the vitamin was given parenterally. The thin line represents the capillary resistance in cms. Hg negative pressure as measured by the suction cup method.

Observe the prompt and extreme response of the capillaries the first day, the immediate recession and gradual increase thereafter. This phenomenon has frequently been observed by us. The arrows indicate that the capillary resistance exceeded the limits of the apparatus i e., was greater than 65 cm. Hg. The drop in resistance on the 23d day has not been correlated with other features of the case. Observe that under the influence of massive doses of vitamin the capillary resistance was double what seems to be normal for the man. The time relationship between vitamin intake and capillary resistance is approximately the same during increasing and decreasing dosages.

The urine contained 0.4 mg. per cent and the blood less than 0 1 mg. per cent on admission. As measured by urinary excretion this man remained unsaturated for more than two weeks despite a total intake of 3.5 *gms* of ascorbic acid However his urine was always alkaline and the actual values may have been higher because of this effect. Symptomatic recovery commenced on the fifth day and was complete on the tenth.

even opposite sides of the body. It is not regularly related to blood plasma vitamin C (Abt, Farmer and Epstein), nor have all investigators found a relationship between saturation and resistance. However this is less important than a lack of relationship between *scurvy* and resistance which has not been proven to exist.

A thorough trial of the capillary test as a measure of vitamin C deficiency in groups of children has been reported by Roberts, Blair and Bailey.

Their report is recommended both as being a thorough trial of the test and a good review of the experience of others. A distinct, statistically significant correlation was found between season, capillary resistance and ascorbic acid intake. The differences between the children on an institutional diet and those receiving supplements of vitamin C (bananas) are shown in table 35, page 294.

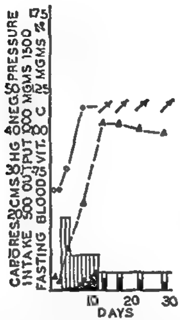

FIG. 40 Rate of recovery of normal blood concentration of vitamin C, capillary resistance, and urinary excretion of vitamin following oral administration of 400 mg. of vitamin C as orange juice Also showing maintenance of normal values on 100 mgm of vitamin. The solid line represents the capillary resistance values, the broken line the concentration of vitamin in the blood plasma, the hollow columns the intake, and the solid columns the amount excreted in twenty-four hours. The patient suffered from hyperemesis gravidarum, and had epistaxis and hemorrhagic gums According to the history her diet has been adequate until six weeks before treatment, the deficient diet was extremely restricted. (This and the following four figures are based on cases studied at Grasslands Hospital by Dr. Ruell A Sloan. See J. Lab & Clin. Med. 23 1015, 1938. Reproduced by permission of the author.)

The virtue of the capillary test is that it is a measure of scurvy and capillary fragility due to vitamin C depletion, as identified by a test dose of vitamin C and followed by observations of the resistance is prima facia evidence of a *pathological* degree of depletion. This the chemical tests do only by inference. There is no reason to believe that it is precise or uniform to any greater degree than other measurements of body function and much of the criticism of it has come from individuals who have looked for a degree of precision which the test lacks. The method of reading the

results should reflect this limitation and the small differences between individuals overlooked in a search for distinctly abnormal, pathological responses.

Capillary resistance, blood and urine ascorbic acid, and saturation tests have all been studied in our laboratories. Typical results were reported by Sloan.

Of the single determinations the fasting blood level proved to be the best index to the patient's nutrition. The single test of urinary vitamin C is frequently very misleading. This may be improved by following Vauthey's suggestion of estimating the "basic ascorburia" (milligrams ascorbic acid per cubic centimeter urine of the excretion within one hour after the first matutinal micturition). Vauthey considered this to be a

TABLE XXXV

GROUP	NO FRAGILITY PRESENT	FRAGILITY PRESENT
	per cent	*per cent*
Control	32	26
Treated	53	5

The blood ascorbic acid values for the same groups were:

GROUP	1 MGM. PER CENT OR MORE	LESS THAN 0 7 MGM. PER CENT
	per cent	*per cent*
Control	13	64
Treated	38	26

physiological constant. It would seem to be the best method of judging vitamin C balance by a simple urinary determination.

The most precise method of estimating the status of an individual is by determining the blood curve following the injection of a standard test dose. This and other results are shown in figures 39–44 inclusive, based on Sloan's studies. The rate of excretion after a test dose is satisfactory in most cases. Orginally the 24 hour excretion was measured. Equally reliable results may be secured by measuring the excretion during a 3 hour period.

Smith has suggested calling the capillary resistance test a measure of the physiologically indispensable intake, the maintenance of uniform excretion following saturation, a test of adequacy, and the maintenance of saturation a measure of saturation or luxus consumption. This seems to accurately epitomize these procedures. The following correlations between such tests is based largely on our own experience.

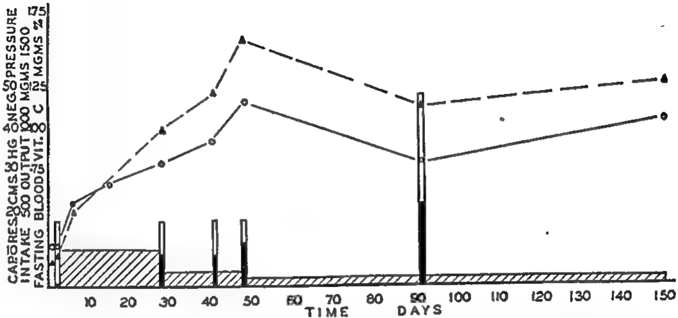

FIG. 41. Progressive increase in fasting blood concentration of vitamin C, capillary resistance and urinary excretion following alternating oral and parenteral administration of the vitamin. The shaded columns represent the vitamin C value of orange juice. The other values are shown as in the preceding figure.

Saturation. Best determined by blood curves following a test dose is regularly accompanied by a capillary resistance above 40 cm. Hg negative pressure as determined by the suction cup technique or by a negative test by Göthlin's *tourniquet method.* The urinary excretion following a test dose is greater than 40 per cent within 3 hours of administration. The blood curve is elevated throughout the test period. The blood plasma values are almost invariably above 0.8 mgm. per 100 ml.

Partial Depletion shows intermediate values in blood curves, urinary excretion and capillary resistance. In most cases the latter will be positive

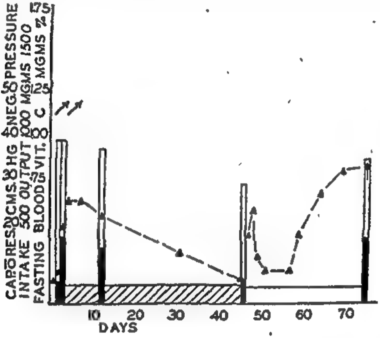

FIG. 42. Failure to maintain saturation and normal blood values of vitamin C on oral intake of 250 ml. of orange juice daily. Recovery on the same amount given parenterally (following the forty-fifth day). The patient suffered from peptic ulcer and had severe hemorrhages previous to treatment with vitamin C. Falsely negative capillary resistance test due to anemia.

in the range of 15 to 35 cm. Hg negative pressure. The blood plasma values may vary from 0.2 mgm. per 100 ml. to nearly 1 mgm.

Depletion reveals prompt absorption of a test dose from the blood stream, the curve falling within two hours to subnormal values. Little or no vitamin C is excreted. The blood plasma values are less than 0.2 mgm. per 100 ml. The capillary resistance is very low, usually innumerable petechiae occur at less than 10 cm. Hg negative pressure. The tourniquet test is strongly positive.

In any of these groups the chemical tests measure depletion, the capillary test, if it can be shown to respond to treatment, a morbid consequence of

depletion, i.e., scurvy. In a strict sense the chemical tests cannot be used to diagnose scurvy but only to rule out that diagnosis for while scurvy has never been reported in an individual without chemical evidence of depletion ascorbic acid may be present in amounts less than 0.1 mgm. per 100 ml. without any symptoms or signs of scurvy (including capillary fragility) associated. Instances of this sort are becoming more and more numerous. Kajdi, Light and Kajdi emphasize this discrepancy and its frequency. The cases described earlier in the discussion of the incubation period of scurvy are excellent examples.

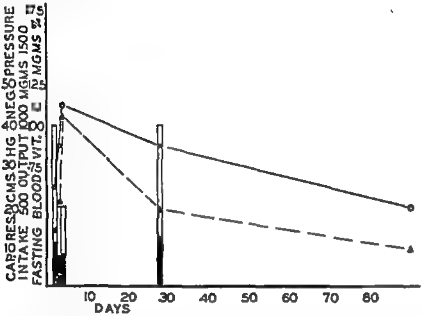

FIG 43. The immediate response to large doses of vitamin C as shown by capillary resistance tests and blood concentration of vitamin and the subsequent fall in both values during a period in which the patient was maintained on a diet poor in vitamin C.

For everyday clinical problems the fasting plasma level is generally used. If the values are normal scurvy can, of course, be excluded. Rinehart and Greenberg offer very practical advice on its interpretation. They have found that 80 per cent of patients having a fasting plasma ascorbic acid level of less than 0.1 mgm. per cent show clinical improvement when given vitamin C and may therefore be presumed to have scurvy. Among those whose values were between 0.1 and 0.3 mgm. a smaller number responded. Values above 0.3 mgm. per cent they found sometimes associated with tissue depletion but not with symptoms amenable to treatment with ascorbic acid. The most common symptoms of these borderline deficiencies were lassitude, anorexia, fatigue and rheumatic pains.

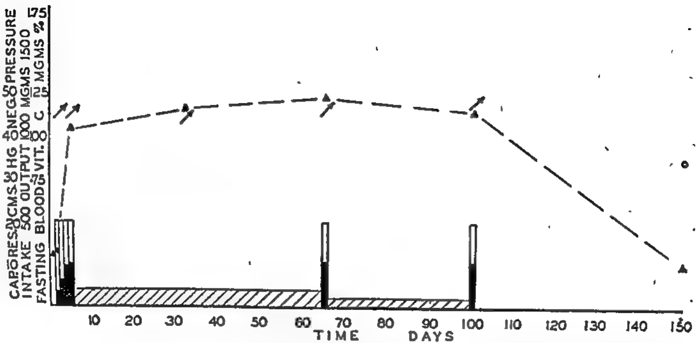

Fig 44. The rate of saturation of an individual given large doses of vitamin C as shown by urinary excretion and rise in blood content of vitamin, the maintenance of saturation on a diet supplemented with orange juice and the subsequent depletion when the patient returned to his home.

Butler and Cushman found that ascorbic acid persisted in the blood cells after the plasma was depleted and recommended that the concentration in the buffy layer of centrifuged blood be used. Their results in experimental deficiency were very encouraging.

Two other procedures may be mentioned. Rotter's test consists of injecting 0.1 ml. of 1:400 dilution of 2:-dichlorophenolindophenol intradermally and timing the period of decolorization. During depletion the dye remains visible for more than 10 minutes. It has been endorsed and criticized. We have not used it. On the other hand radiographic diagnosis is often useful. Improved criteria were described by Park, Guild, Jackson and Bond.

TREATMENT

The treatment of scurvy requires only the administration of ascorbic acid. This may be given by mouth, intravenously or intramuscularly. Several grams are required to saturate a severly depleted, scorbutic individual. In such cases the vitamin is best given in gram or half gram doses and intravenously. The dose may be repeated daily or on alternate days. Response occurs very quickly. Goettsch has demonstrated that single, massive doses are as effective in scurvy as divided doses and that signs of repair may occur within 48 hours (calcification of subperiosteal hematomata).

For less severe deficiencies smaller doses are adequate, say 100 mgm. daily.

Much is still to be learned by the study of cases of scurvy. The apparent requirements of the particular case, the amount necessary to cure, factors which predispose to scurvy and related disturbances and lesions are all matters of lively interest. For these saturation tests, a basal diet of known ascorbic acid value and other clinical tests and measurements are valuable.

Hypervitaminosis

Toxic symptoms have not been produced in animals by ascorbic acid. Doses of 1 to 6 gm. are well tolerated by man. Widenbauer noted vagotonic symptoms but ascribed them to idiosyncrasy.

CHAPTER XXII

VITAMIN D DEFICIENCY

Vitamin D is distributed much as vitamin A. The provitamins occur in plants and the physiologically active forms are only formed in animal tissues. In the case of vitamin D the provitamins are activated in the skin by ultraviolet light.

The great importance of vitamin D in clinical medicine is in the calcification of bone. Since the infant requires a very rapid rate of calcification to keep up with bone growth the requirements of vitamin D are especially imperative at that time of life and the effects of deficiency most rapid and severe.

The lesions of rickets are due to diminished calcium or phosphate ion concentration in the blood. One might say therefore that rickets is primarily a disease of the blood for its most important function seems to be in the absorption of calcium from the bowel. However there are old and valid experiments which indicate that vitamin D also has an immediate and direct effect on the bone. Rachitic bone will calcify when immersed in normal serum (Hess) and the addition of calcium and phosphate to rachitic serum will not do so. Shohl's discussion is recommended.

The requirements of vitamin D are not known excepting as clinical experience has established 400 to 800 Int. Units as the desirable intake for infants. The requirements of other species vary tremendously. The rat, for example, is resistant to measures which cause rickets in man. The efficacy of the various antirachitics varies a great deal between species.

HISTORICAL

Rickets was prevalent in England for two hundred years before it was first accurately described by Whistler (1645) and Glisson (1650). It was long known on the Continent as the English Disease. Whistler's book, published during his studies in Leyden, was for many years forgotten and the medical history of rickets is usually said to start with Glisson's monograph. It was undertaken through the joint interest of a committee of the College of Physicians. Glisson made no reference to Whistler's work nor Arnold Boate's text in which the disease is clearly described and which was published a year before Glisson's "De Rachitide." A full and fascinating account of these matters appears in Drummond and Wilbraham's "The Englishman's Food."

None of these authors recognized the dietary nature of rickets. Sir

PERMANENT DECIDUOUS

NAME (Mother) Date)

Address Tel

Sex Color Date of Birth

Prev. Attacks No Pregnancies

Operations Accidents

Wrist X-ray Dental X-ray

Grid Physique

ORAL MUCOUS MEMBRANES—DEGREE OF REDNESS

Gums U L	B & L MM U L	Bl Ves. dil	Inside Lips	Lips	Floor	Palate Hard Soft	Cheek	Ulcers

PERIODONTAL

Gingivitis	Pockets	Pus	*Calculus*	*Detritus*	*Mobility*	*Bone Loss*	*Vincents*

DENTAL

CARIES Deciduous Permanent	Missing Teeth	Reason Lost	Eruption	Arch Form	Occlusion	Care	Restorations

MEDICAL

R ANGLES L					TONGUE						
Season	Onset	Crust	Scar	Wet	*Papillae* T S B	*Redness* T S B	*Fissures*	*Edema*	Teeth Marks	*Ulcers*	*Coated*

EYES				MENTAL		PAIN				
Conj P B	Lacrimation	Burning	Night Blindness	Nervous	Hallucinations	Arm	Leg	Head	Stomach	Teeth

SKIN				TASTE	NOSE	HAIR					
Burn	*Pigment*	*Dermatitus*	Site				*Appetite*	*Constipation*	*Diarrhea*	*Weight Loss*	*Tire Easy*

DIET

Meat	Vegetables	Bread	Desserts, Sugars	Economic Status
Lean	Irish Pot.	Cornbread	Desserts	Brothers
Fat	Sweet Pot.	Biscuit	Sugars	Sisters
Liver	Beans	Loaf	Syrup	Urban Rural
Fish	Turn Greens	Cereals	Jam, Jelly	Land
Fowl	Fruits	Cornflakes	Candy	Rent Share Own
	Oranges	Oatmeal	Milk	Income
	Others	Grits	Eggs	Chicken Hogs Cows

BREAKFAST DINNER SUPPER

Key to abbreviations
B & L MM —Buccal & Lingual Mucous Membrane
Bl Ves. dil —Blood Vessel Dilatation
Papillae T S B—Tip-Sides-Body
Redness T B S (same)
Conj P B—Conjunctiva Palpebral, Bulbar

DIETARY DEFICIENCY

Niacin	Thiamin	Riboflavin	Iron	Vit. A	Vit C	Vit. D	Protein	Fat	Carbohydrate

FIG. 45 A form for the convenient recording of common signs of deficiency disease and pertinent dietary history. (Courtesy of Dr. Tom. D. Spies.)

Thomas Browne, in 1672, wrote of ravens that "many are killed for their Livers, in order to the cure of the Rickets." By 1882 Trousseau in his "Clinical Medicine" recommended cod liver oil as a cure of rickets and a few years later Palm associated the incidence of the disease with the prevalence of sunshine. Park refers to Buchholz as having cured rickets in 1905 by artificial light. But none of these opinions were widely known nor did their discovery influence the prevalence of the disease.

The modern history of rickets commences with Edward Mellanby who by 1919 succeeded in producing rickets in puppies by dietary means and curing them with animal fats. The following year Sherman and Pappenheimer and McCollum and Simmonds induced rickets in rats. In 1921 Hess and Unger confirmed the curative power of sunlight on both human and experimental rickets. The fat soluble vitamins were separated by saponification and the activation of sterols demonstrated. By 1927 it was demonstrated that the impurity in the sterol which was activated was ergosterol which, curiously enough, had been isolated in 1811 by Braconnot and rediscovered in 1889 by Tanret.

FREQUENCY OF VITAMIN D DEFICIENCY

Crippling rickets has become uncommon or rare within the past twenty years in North America and persists only in the presence of great poverty, ignorance or where religious practices impose seclusion on women (the Hindu purdah). Sebrell's reports of incidences of as high as 97 per cent in Europe and North America are based on radiographic examinations of the bones and the interpretation of such evidence is subject to great personal bias in marginal cases. There is indeed no clear-cut way by which extremely mild rachitic lesions can be objectively measured. The number of deaths from rickets in 1941 was 139 according to the U. S. Bureau of the Census, having declined steadily since 1933. Osteomalacia is relatively common only in India and the Far East.

PREDISPOSING FACTORS

Vitamin D deficiency most conspicuously affects mineral metabolism and most characteristically and importantly results in rickets. But in two respects this avitaminosis differs from others. In the first place dietary intake is supplemented and may be largely replaced by endogenous synthesis. In the second place the effect of the deficiency is intensified or minimized by mineral supply and this is dependent quite as much on intake, requirements and the function of other organs as on vitamin D. Therefore the vitamin is not always of decisive importance.

The synthesis of vitamin D in the body occurs in the superficial layer of the skin through the action of certain solar rays. This has been demon-

strated by the irradiation of excised portions of skin (Luce-Clausen). The first effect of its formation, or its ingestion, is an increase in blood phosphorous which is followed by lime salt deposition in the bones. Vitamin D presumably has nothing directly to do with the latter but functions only to increase calcium absorption from the intestine or to increase phosphorous retention which in turn increases calcium retention. Naturally calcium and phosphorous must be present in adequate amounts as well as certain organic acids. (Shohl).

In practice, however, the mineral intake is much less important than the supply of vitamin D and the effect of the latter is so great that relatively unfavorable mineral conditions may be corrected by adequate amounts of

TABLE XXXVI

The Mineral Metabolism in Rickets

	MINERAL METABOLISM		CLINICAL SIGNS
	PHOSPHOROUS BALANCE	CALCIUM BALANCE	
Phase 1	Strongly negative	Normal or nearly so	Asymptomatic or very early rickets
Phase 2	Strongly negative	Negative	Symptomatically manifest rickets recognizable radiographically
Phase 3	Positive (more positive than normally)	Negative	Clinical and radiographic signs of recovery
Phase 4	Postive	Positive (more positive than normal)	Clinical evidence of advanced healing. Broad bands of calcification in radiographs

After Rominger, E Ergebn d Vitamin u Hormonforsch., 2. 104, 1939.

vitamin D. Nevertheless experimental rickets is induced not by vitamin deficiency alone but by vitamin deficiency plus a relative deficiency of calcium or phosphorous or an absolute deficiency of either or both. Spontaneous rickets is usually a low phosphorous rickets.

We have mentioned that vitamin D is effective only through the mechanisms of mineral metabolism and therefore dependent upon it. This is best demonstrated by reference to rickets caused by feeding cations which by forming insoluble phosphates intercept calcium in its progress to the bones. Beryllium will do this as Branion, Guyatt and Kay have shown in rats. It is the probable explanation of Caffey's case in which an infant receiving adequate amounts of cod liver oil but suffering from lead poisoning developed rickets. An interesting relationship between the

metabolism of lead and calcium is seen in cases of this kind since the usual lead deposit in the bones is missing just as calcium is.

Nephrosclerosis is associated with an uncommon condition in infancy in which a mineral disturbance and lesions resembling those of rickets occur. The theory has been proposed that phosphorous is retained in these cases and that low blood calcium is due to high blood phosphorous (which in contrast to dietary rickets is regularly present). Mitchell, however, suggested that phosphorous retention increases the intestinal excretion of phosphates and that these precipitate intestinal calcium and prevent its absorption. Pappenheimer demonstrated that a low calcium diet fed young rats in which kidney tissue had been reduced in amount produces a stunted growth and skeletal lesions resembling "renal rickets." Many cases of "renal rickets" are due to hyperparathyroidism.

If these are excluded there remains a syndrome most common in the early teens which affects both sexes and which usually terminates fatally before the 20th birthday. Genu valgum is frequent. The radiographs reveal typical rachitic lesions. The common symptoms are due to the kidney disease, polyuria, nocturia, headache and loss of appetite. Laboratory studies show diminished kidney function, azotemia and a reversed calcium-phosphorous ratio. In addition the subjects are distinctly dwarfed. The parathyroid tissue is hyperplastic. This has been ascribed to the influence of the retained blood phosphorous which the parathyroid controls. Whatever the explanation of the association of renal, osseous and parathyroid lesions may be it is close and evidently much more frequent than has been suspected. Thus Follis and Jackson found that 9 of 12 patients who had died of glomerulonephritis, 3 who had died of pyelonephritis and 7 of 24 who had died of vascular nephritis had excessive osteoid deposits in their vertebrae. Osteitis fibrosa was not encountered excepting rarely and in scanty degree. The parathyroids were not examined. Following accepted terminology this condition would be called *renal osteomalacia.*

Mention must also be made of the views of Mellanby who has emphasized the significance of certain cereals in the production of rickets Mellanby's views arose from the observation that cereals varied greatly in their ricket-producing qualities and that those with higher phosphorous and calcium content, such as oatmeal and wheat germ, were more liable to produce rickets than rice and white flour. Mellanby has ascribed these reactions to the presence of an "anti-calcifying toxamin." A more popular opinion is that experimental diets predominantly containing such cereals induce rickets because they contain phosphates in the form of phytin which is poorly absorbed. This in no way detracts from the significance of Mellanby's views to the present discussion and serves equally well to demonstrate how other factors, of which perhaps but a few are yet known,

determine vitamin requirements and whether lesions ordinarily ascribed to vitamin deficiency do or do not develop despite "adequate" intake.

In 1933 Gerstenberger reported cases of rickets in infants suffering from biliary obstruction (congenital obliteration of the ducts). These were refractory to cod liver oil treatment. The circumstances have been reproduced in rats by Heymann both by ligation of the bile ducts and liver necrosis from carbon tetrochloride poisoning. Under such circumstances the requirement of vitamin D, as judged by the cure of rickets, is increased from 10 to 12 times the normal amount. Whether this is wholly due to faulty absorption through lack of bile or whether the liver operates in some other manner is not known.

Other forms of rickets have been described which are refractory to vitamin D. These have lately been cited by Park. In certain cases the refactory state persists for long periods. Albright, Butler and Bloomberg have reported the natural history of such cases including one observed for 14 years. The failure to respond appeared due to an intrinsic resistance to vitamin D since the refractory state remained after absorption had been circumvented by intravenous medication and ultraviolet radiation. In some of these cases at least the resistance to vitamin D effect is only relative, as is true of so many related deficiency diseases, and can be overcome by large doses.

Rickets proved to be a complication of two cases of chronic acidosis studied by Boyd and Stearns and was ascribed to the chronic base deficiency.

THE MORBID ANATOMY OF VITAMIN D DEFICIENCY

Pathologic anatomy has been of inestimable value in studies of vitamin D deficiency. The anatomical criteria are easily recognized and have served to guide many valuable experimental studies. Rickets was well understood, from an anatomic standpoint, long before the period of vitamin research and the older knowledge of rickets had only to be applied to the induced forms of the disease in dogs and rats to afford accurate means of measuring the effects of various diets and antirachitic agents.

The anatomic difficulty today is largely dependent on the fact that similar lesions occur in other periods of life than infancy and under various conditions. In such cases they are frequently too imperfectly formed to permit precise diagnosis. The problem today is one of determining the rôle of vitamin D in such borderline states and the anatomic approach to this problem has yielded relatively little new information.

At least three diseases share the pathologic anatomy characteristic of deficiency of vitamin D. They are rickets, by far the most common of the conditions, adolescent rickets and osteomalacia. Pathogenetically, histogenetically and etiologically no fundamental differences exist between

them. Combination forms are not uncommonly seen in animals. In such cases the bones which have ceased to grow, for example the bones of the extremities, develop osteomalacic lesions while the still growing ribs become rachitic. Late, or adolescent rickets frequently constitutes a similar transitional state in man.

The gross differences between rickets and osteomalacia are due to the presence or absence of growth in the affected bones. Growth, furthermore, determines the degree of the rachitic lesions, the two being strictly proportional. The most apparent rachitic lesions, at sites of enchondral bone growth, are lacking in osteomalacia simply because enchondral bone growth is lacking. The relative paucity of osteoblasts in osteomalacic lesions is due to the normally fewer osteoblasts in the bones of adult animals. The tendency for fractures to occur in osteomalacia while bending is the rule in rickets and is due to the persistance of some rigid, calcified bone in the former, a survival from healthier days and the natural difference in rigidity between the bones of infants and adults.

Rickets and osteomalacia share another significant feature. Both are closely related to normal physiological tides. Rickets is an exaggeration of changes which usually occur in the winter months and represents an imbalance of forces constantly at work in growing bones. Schultz, for example, has insisted that the experimental criteria of a normal costochondral junction are false and represent a stage of over-dosage of vitamin D. In the case of osteomalacia the process seems pathological only in the degree to which it exceeds the normal changes in pregnancy. Whether normal in either case is identical with optimal conditions is, of course, open to serious doubt but the point remains that both processes represent exaggeration of disturbances of bone growth which are the rule among our people.

The confusion which still exists concerning the pathogenesis of osteomalacia seems to us to be due to a number of causes. In the first place the disease is relatively infrequent and has not been studied as thoroughly as rickets. In the second place precise diagnostic methods have seldom been applied to cases of osteomalacia. The clinical attitude has been that unquestionable cases are those instances of softening of the bones during adult life without definite accompanying bone disease. By these standards other conditions and especially osteitis fibrosa must frequently have been confused with osteomalacia. The third cause for confusion has been the successful treatment of many cases by castration, adrenalin and pituitary extracts. The former is said to be effective in almost all cases and reversal of the negative calcium balance has been observed following bilateral oophorectomy.

Too many glands besides the parathyroids have been implicated to

maintain the theory that these cases are due to hyperparathyroidism. Moreover the chemical studies are in complete accord with the known facts concerning rickets, reduced plasma phosphorous, increased phosphatase etc. We believe therefore that osteomalacia is essentially the adult counterpart of rickets but that other osteo-dystrophies have frequently been confused with it and that, in the past, a variety of conditions have masqueraded under the title of osteomalacia. The problems associated with the pathogenesis of osteomalacia will probably not be fully solved until the association of parathyroid hyperactivity and rickets is solved.

Blumgart, Gargill and Gilligan came to the same conclusion after intensive metabolic study of a case of osteomalacia. The mineral balance, the retention of calcium and phosphorous when vitamin D or ultra-violet light was given was similar to that occurring in rickets. The hematologic and radiographic features of their case were also closely related and they concluded their study with the comment that "osteomalacia, as manifested by this patient, is a form of adult rickets."

In a recent essay Mellanby reports the production of osteomalacia in dogs by dietary means. The photographs of the ribs are very convincing but other data are not given.

Of the other conditions which share the histologic lesions of rickets the most interesting is deficiency osteoporosis or "hunger osteopathy," a disease which became conspicuous in Vienna during the World War. The similarity of this condition to osteomalacia emphasizes the unsatisfactory terminology employed for these bone diseases all of which seem to be essentially the same.

In deficiency osteoporosis the bone pains of osteomalacia are prominent and are associated with similar, if less well marked, changes in the long bones. Bending and fracture of bones may occur. The cases reported seem to have been the late effects of sharply restricted diets. They respond favorably to cod liver oil. It would seem that these cases might be considered as transitional forms between osetomalacia and senile osteoporosis.

The lesions in spontaneous rickets in man and other mammals are frequently very complicated due to secondary factors such as stresses and strains and to variable dietary and hygienic effects. Such lesions are naturally less suitable than experimental lesions for purposes of demonstration. For a discussion of the lesions of human rickets Pommer's monograph remains a classic contribution and the monograph by Marek and Wellman expands the field by including studies of the lesions in a variety of domestic animals as well as man. The purposes of this book will be served by a description of the morbid changes in experimental

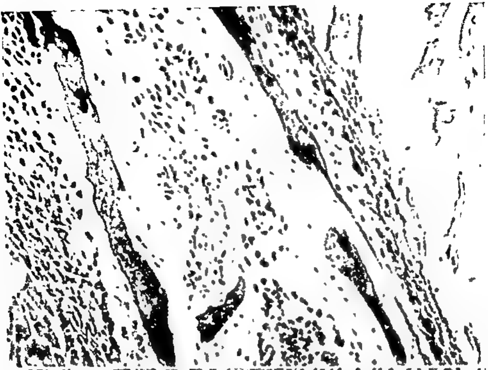

Fig. 1

Fig. 2

PLATE XLI Rachitis Figure 1 from the rib of a rachitic rat The great quantity of homogeneous, uncalcified osteoid tissue is seen in prolonged rickets. Figure 2 is from a similar animal and shows the appearance of the redundant cartilage masses which form in vitamin D deficiency

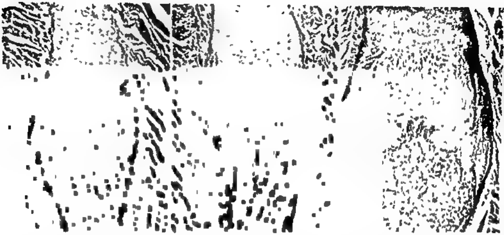

PLATE XLII Experimental rickets Three stages in the development of rachitic lesion at a costo-chondral junction. Photographs made at same magnific tion from animals of same size and on same diet but examined at increasing period Notice the overgrowth of cartilage and osteoid tissue within the bone and abou the periosteum *Notice the increasing size of the junction which is responsible fo the rachitic "rosary"*

thin. Dodds and Cameron have described the metaphysis in rickets in detail. They consider the lesions to be characteristic and important. The tissues corresponding to the primary spongiosa of normal bones are formed of cartilage remnants on which are deposited considerable amounts of osteoid. These masses of osteoid may be identified by the uncalcified cartilage matrix within throughout the healing process.

The first sign, of healing are the deposition of calcium in the cartilage, usually seen within twenty-four hours after giving cod liver oil (Pappenheimer) and the reappearance of clear, degenerative cartilage cells on the diaphyseal side of the cartilage plate. Wolbach states that this effect is also noticeable within twenty-four hours. Lime salts are also promptly deposited in the osteoid material. The capillaries commence to invade the degenerate cartilage (within forty-eight hours, Wolbach) and the formation of natural bone trabeculae occurs.

Calcification, in recovery, does not develop uniformly but in scattered foci. Hess stated that the earliest calcification appeared not at the metaphyseal margin of the redundant cartilage but within it, where the normal zone of calcification should be. Dodds and Cameron disagree with this. They found the earliest calcification in the metaphysis and that it reached the level designated by Hess only after healing was well advanced. They also stated that a characteristic phenomenon of healing rickets was that calcification first spreads toward the shaft in a reversal of the normal direction. Only when healing is far advanced is the direction restored to normal.

An early sign of healing is the invasion of the degenerating cartilage by capillaries which occurs within 48 hours of treatment. This erosion commences from the lateral margins. The deposition of lime salts is the basis of the commonly used "line test" in which the bone is soaked in silver nitrate solution and then exposed to light. The calcium deposits so revealed are routinely used in gauging the presence and extent of healing.

Rachitic bones appear to be enlarged near the joints but this may be only a relative enlargement due to cessation of growth of the adjoining cortex. Dodds and Cameron support this view with measurements of rachitic and normal rat bones. The chemical composition of the rachitic bone is similar to that in scurvy. The amount of ash is less but the calcium phosphorous ratio is normal indicating that if these minerals are deposited at all they are deposited in the usual form. Growth of long bones is retarded very early in rickets but continues at a slower rate for three weeks when it ceases altogether (Dodds and Cameron).

Histologic aids in the diagnosis of rickets include the presence of *excess* osteoid tissues, best recognized in the shaft. "Presence of osteoid in excess is a cardinal sign of rickets and is pathognomonic of the disease. It

is the only sign in osteomalacia and may be the only sign in the older child" (Park). But rickets may be present without excess of osteoid to indicate its presence. Failure of lime salt deposition is an early sign. This may be only focal, consisting of gaps in the normal line of calcification. Distorted or crushed cartilage cells and excess cartilage cells should be searched for. The general structure of the metaphysis is that of great disorder due to irregularities of growth and the complications caused by stresses in an insecure organization.

The teeth in experimental rickets reveal the same lack of calcification seen in the bones. In this case matrix continues to form, but is not naturally calcified. One of the earliest changes is that the globules of lime are reduced in size, and uncalcified spaces appear in the dentine. Except in severe cases, the odontoblasts are not affected as far as can be judged from their appearance. The earliest changes in the teeth of rats occur within the first week according to Becks and Ryder, and consist of a distinct widening of the predentinal layer. The layer is irregularly filled with globular masses of lime salts. Subsequently, calcification ceases entirely, and the matrix occasionally is seen invaded by small vessels, a feature which is uniformly present after prolonged rickets. Becks and Ryder consider that the primary effect of the deficiency is on the odontoblasts, not due to lack of lime salts, but rather to lack of vitamin D, and by analogy they feel that the same is true of the lesions in the bone.

The relationship between dietary vitamin D and dental structure has obvious importance and studies of this relationship in man and their bearing on the problem of caries is discussed elsewhere in this volume. It is noteworthy that Wesson and Boyle have found that vitamin D improves bone and tooth structure in the rat *independently of its effect on calcium retention*. The effect of vitamin D on the bones of the skull and the teeth is illustrated in Plate XLIII.

In Mellanby's original observations of experimental rickets hypertrophy of the thyroid gland was described. A wide variety of studies have since been made of the condition. Thompson has said that the hypertrophy of the thyroid gland in rickets was due to dietary deficiency in iodine and could be prevented by feeding small amounts of iodine. This hardly explains other results. For example Bergman showed that active secretion developed in rats raised in darkness and that the secretory phase changed to one of colloid storage when they were exposed to light. Nitschke demonstrated a similar effect of viosterol on the thyroid. These must have been due to vitamin D.

Nitschke and Doering explored other phases of the problem. They found infant rickets regularly accompanied by low blood iodine concentrations which returned to normal after 10 to 20 days of treatment. In florid

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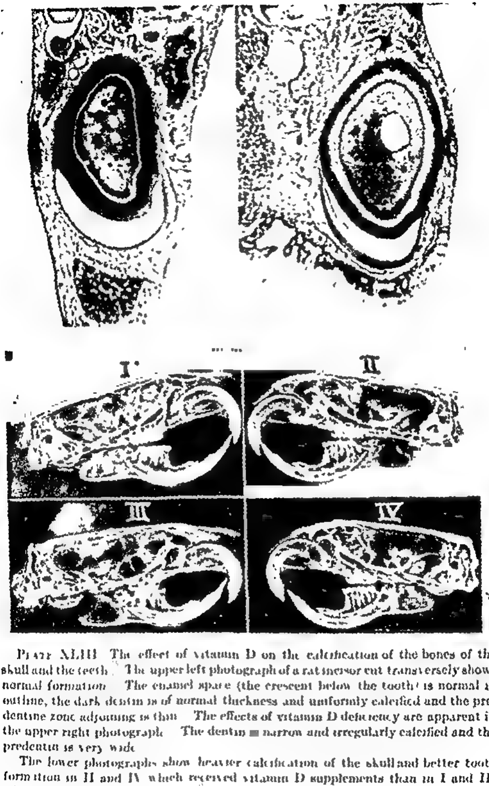

PLATE XLIII The effect of vitamin D on the calcification of the bones of the skull and the teeth. The upper left photograph of a rat incisor cut transversely shows normal formation. The enamel space (the crescent below the tooth) is normal in outline, the dark dentin is of normal thickness and uniformly calcified and the predentine zone adjoining is thin. The effects of vitamin D deficiency are apparent in the upper right photograph. The dentin is narrow and irregularly calcified and the predentin is very wide.

The lower photographs show heavier calcification of the skull and better tooth formation in II and IV which received vitamin D supplements than in I and III which were fed the deficient diet. (Courtesy of Dr. Paul E. Boyle.)

rickets the concentration of iodine in the blood fluctuated between 2–5 γ per cent, lower values than occur in myxoedema. In normal children the value was between 7.5–13.5 γ per cent. These authors also state that thyroxin intoxication is associated with an increase in blood phosphates indicating that a close relationship exists between phosphates and thyroid function.

The parathyroid glands are enlarged and hyperplastic in experimental rickets, in rachitic infants, and in osteomalacia. Erdheim's original observations have been repeatedly confirmed. Various explanations for this association of parathyroid hypertrophy and rickets have been suggested. The concensus seems to be that the glandular enlargement is compensatory and plays no significant rôle in the rachitic process. Ham and Lewis have recently explained related experimental results by considering the action of vitamin D as intermediary in the parathyroid mechanism. The point is unsettled. Several significant reports may serve to indicate the complexity of the problem.

Chicks deprived of vitamin D to a degree insufficient to produce rickets develop parathyroid hyperplasia. On the other hand a partial deficiency of parathyroid hormone intensifies a deficiency of vitamin D and increased parathyroid hormone reduces the requirements of the vitamin. But of greater significance than these interesting quantitative studies is the demonstration of Pappenheimer that vitamin D is antirachitic in parathyroidectomized rats. The rôle of these incretory glands is evidently of secondary importance.

The most recent contribution to the subject are the rat experiments of Ham et al. which seem to show that parathyroid hypertrophy is not a feature of rickets per se but only of low calcium rickets. In their animals low phosphorous rickets was not associated with hypertrophy. Moreover hypertrophy was produced by diets which caused the blood calcium levels to remain low, in the absence of rickets, but not by diets which caused high blood phosphorous values.

EXPERIMENTAL RICKETS AND CONSTITUTIONAL FACTORS

The lesions in experimental rickets are less constant than those in other deficiency diseases. The variable susceptibility of individual animals has been credited to constitutional differences. In some cases the maternal diet has been responsible. In Grant and Goettsch's investigation of this problem it was discovered that female rats could be depleted of their vitamin stores and that once this had occurred their litters developed rickets more rapidly and to a greater degree than normal animals. Similar results have been reported by the Toveruds in dogs. That this mechanism, of maternal nutrition, does not explain all of the cases of susceptibility or

resistance to rickets has been shown by Hess and Blackberg who fed identical diets to four puppies from one litter. Two of the mongrels were short haired and two long haired and other constitutional differences were evident between the two pairs. The two short haired terrier dogs developed more severe rickets than their litter mates. Stockard has observed constitutional differences among dogs and a pronounced difference between sexes. According to Stockard it is difficult to raise male St. Bernards with normal skeletons while the females seldom develop rickets.

THE MORBID ANATOMY OF HUMAN RICKETS

The rachitic deformities depend on the mechanical influence of the weight of the body on the weakened bones. The diaphyses bend outward in active children, the femurs also arching forward as well as laterally. Extreme bending can also occur near the joints and result in such deformities as genu valgus. Angulation in the soft osteoid tissue leads to common chest deformities in which the costo-chondral junctions are sharply depressed and the sternum pushed ventrally to form a "pigeon breast."

The skeletal lesions vary in their gross appearance with the extent of the disease and its distribution. If the periosteal and endosteal osteoid tissue is extensive the bone cortex is soft, pinkish in color and easily cut. In other cases the cortical changes are absent or insignificant. The epiphyseal lesions, once they are transected, show the characteristic chondral overgrowth and the heavy osteoid masses which fill the end of the marrow cavities. The skull may show osteoid masses growing beneath the periosteum as well as large fontanels and defects in the skull bones.

The histologic changes need not be given. They would only recapitulate what has been said of the bone lesions in rat rickets.

The lesions in human teeth are seen in the permanent dentition since rickets occurs during the calcification of these teeth. Dimpling of the enamel, furrows, wave-like and point defects occur (Freudenberg). The available evidence all points to the important rôle of vitamin D in the development of the teeth (Schultz) though it should be remembered that the effects of vitamin A deficiency on the enamel organ have been relatively neglected in the studies of human dentition and some effects ascribed to vitamin D may have been due to vitamin A as well.

Be that as it may the association of rickets, formerly so common, with equally common developmental defects in teeth which were being formed at the precise time when rickets is most noticeable is highly significant.

Hypoplastic defects of the milk teeth are also very common. May Mellanby found only 21 per cent of 1260 deciduous teeth to be normal in structure. The dental defects described by Mellanby are similar but less extensive than those since found in established cases of foetal rickets.

Wolfe's report may be consulted for illustrations of deciduous teeth stigmatized by rickets occurring before birth. The location of the dentin and enamel defects could be correlated with the period at which these parts are known to form and from such information the probable period of intra-uterine life at which the rickets occurred could be estimated.

Enlargement of the heart has frequently been described in autopsy protocols of cases of rickets. This has recently been discussed by Abt who refers to earlier reports. It seems difficult, in the absence of experimental evidence, to sustain the view that the enlargement was due to rickets per se. Similarly the muscle lesions, loss of striations, interstitial fibrosis and atrophy, are seen only in advanced cases and may be due to complicating scurvy or other conditions. They do not occur in experimental rickets.

The skeletal lesions in osteomalacia affect the pelvis, lower spine, and bones of the lower extremities. However, advanced cases show similar lesions in all parts of the skeleton. Bending, distortion and fractures commonly follow the softening. The bones cut easily, appear greatly rarefied in radiographs and are characterized histologically by an overgrowth of osteoid tissue which surrounds, in thick margins, the trabeculae and laminae and lines the haversian canals

HYPERVITAMINOSIS D

The effects of excessive doses of vitamin D on the bone are premature calcification of the cartilage and active bone formations with the result that the spongiosa becomes dense and compact. Calcium deposits also occur in various organs and in the blood vessels. Collazo and his associates studied the effects on bone in detail, and showed the late effects which are dwarfing of the growth of long bones and the production of animals with short extremities but large heads and long tails. If the dose is still greater, from ten to one hundred thousand times the minimal protective dose, rapid loss of weight and death occur. At autopsy, such animals show enormous calcium deposits in the walls of their blood vessels, and high concentrations of calcium and phosphorous in the blood serum. The metastatic calcification of the organs is largely dependent upon the amount of calcium in the diet. Indeed Harris ascribes great importance in the development of the severe effects from excessive vitamin D to the calcium phosphorous intake. Clouse suggests that the calcium deposits are due to the inability of the kidneys to excrete the excessive amounts of calcium and phosphorous absorbed from the bowel under conditions of high mineral intake and excessive vitamin D dosage. Diseased tissues, such as tuberculous lesions and lesions of encephalitis are especially prone to calcification (Levaditi and Po).

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The bone lessions vary a great deal with the age of the animal and the mineral value of the diet. The initial effect is accelerated calcification of the provisional zone of calcification in the cartilage. Later effects include cessation of cartilage growth and lead to dwarfish animals and osteoporosis but, in distinction to the osteoporosis produced by parathormone, the resorbed areas are not replaced by fibrous tissue (Shelling and Asher).

In the teeth the dentin is excessively calcified and the cementum thickened and hypercalcified (Harris and Innes).

The vascular lesions are not specific ones and Vanderveer was unable to distinguish them from those produced by certain drugs. The earliest incrustations with calcium are about the elastic fibers of the media, later the muscle fibers degenerate. Wahlgreen used smaller amounts of cod liver oil and ergosterol, and produced a chronic intoxication which affected the heart muscle. The cells were swollen, the striations indistinct, and some of the muscle fibers ruptured.

By further adjustment of the dose of vitamin D Collazo and Kohler were able to produce nephrosclerosis which progressed to kidney insufficiency and death. Albuminuria and tubular casts were present in the urine. The larger arteries were sclerotic. Other manifestations of vitamin D intoxication of this degree are metastatic calcification, phosphaturia and polyuria as well as hypercalcemia and phosphatemia. According to Collazo and Kohler these effects may be produced at will in various species and in man.

The histologic sequences in the kidney have been reinvestigated by Goormaghtigh and Handovsky who administered calciferol at various levels to dogs. The earliest effect, according to these authors, occurs in the arteriole where smooth muscle cells and certain afibrillar cells which they believe related to the neruomuscular tissue which occurs in the heart, undergo hypertrophy. On the basis of this evidence the effect is considered to be at first stimulating. Doses in excess of 700 mgm., however, produce necrosis of the smooth muscle cells. As a consequence of slightly toxic doses of calciferol and the production of reversible effects in the arteriolar smooth musculature blood pressure rises and the response to epinephrine is exaggerated. It is significant that Goormaghtigh and Handovsky were unable to produce a permanent hypertensive effect by sustained viosterol intoxication. Once administration ceased the tissues underwent complete restitution.

The amount of vitamin D toxic for the rat has been estimated by Bills et al. as 100 times the protective dose. Such amounts gave perceptible evidence of harmful action. Four thousand times the protective dose was definitely injurious and 40,000 times strongly toxic. In terms of human

doses this evidence, if directly applicable, would indicate that perceptible damage might follow 1000 times a curative dose of 3000 U.S.P. units, an amount represented by 30,000 grams of U.S.P. cod liver oil. It is apparent that the danger of overdosage is remote when the usual preparations of vitamin D are used.

Further evidence on this point is afforded us by the experience of Reed and his associates who have used doses ranging from initial amounts containing 200,000 units to 600,000 to 1,000,000 International units per day. Under this regime toxic manifestations occurred in 9 per cent of their cases. Dreyer and Reed state: "Concentrated vitamin D is no more hazardous than many preparations used daily by physicians. To be sure there are precautions to be observed, but the early symptoms are readily recognized even by the patients themselves, and when they appear, administration of the vitamin should be discontinued at once. Usually one or two weeks is sufficient to allow before resumption of the treatment."

Bills describes the symptoms of hypervitaminosis D as follows: A sense of well being and increased appetite changing to nausea and loss of appetite; vomiting, cramps, diarrhea, and frequent urination. Sometimes neuralgia along the course of the mandibular branch of the trigeminal nerve, tenderness of gums and teeth, pain in the muscles and joints, dizziness, muscular weakness, headache, haziness of memory, and occasionally numbness and tingling in the extremities These symptoms are usually but not invariably associated with a hypercalcemia in excess of 15 to 16 mgm. per cent. Discontinuance of dosage and intravenous saline administration bring prompt recovery.

The effects of prolonged administration of large amounts of vitamin D have not been as thoroughly studied. Few reports of toxic effects exist despite the widespread use of irradiated foods, sunlight lamps and vitamin D concentrates. The belief has grown in recent years that no significant hazard is associated with the use of even large amounts of this vitamin and that some of the earlier instances of toxicity were due to toxic products (toxisterol) developed by too prolonged irradiation of ergosterol. No special contraindications to the use of the vitamin are known.

Wells and Holley report the necropsy protocol of a 59 year old man who had received 5,000,000 U S.P. units of viosterol during a 15 day period for the treatment of osteitis deformans (Paget's disease). Heavy metastatic calcium deposits were found in the lungs, endocardium, kidneys, gastric mucosa and focal areas of the skin. In this case the vitamin D dosage alone was not responsible for the tissues in Paget's disease have a great affinity for calcium. It is nevertheless interesting in that the striking lesions of hypervitaminosis D have been seen in man.

PLATE XLIV. Rachitic deformity in an adolescent. (Photograph from collection of Dr. Wm Watters.)

phosphatase determination in 500 cases of rickets. All values above 11 units (method of Jenner and Kay) were considered abnormal. By this standard 84 per cent of the rachitic children showed positive phosphatase tests. Parallelism was demonstrated between the height of plasma phosphatase and the severity of the process and it was thought that phosphatase values increased before other evidence of rickets appeared. However the values alone should not be accepted as pathognomonic of rickets.

A further aid in diagnosis is the roentgenographic examination of the bones, especially the wrist and forearm. A concave metaphysis with frayed margins and poorly mineralized shaft occurs in cases of moderately advanced rickets. The x-ray examination is also suited to study of the progress of the disease. The weakness of this diagnostic measure is that early, slightly developed rickets frequently cannot be recognized and that interpretation is subject to all the weaknesses of other forms of subjective observation.

Diagnosis is not difficult if the case be more advanced. The rachitic rosary, the tumefaction of the junction of cartilage and bone of the ribs due to the overgrowth of osteoid tissue and cartilage, may be seen or felt, the anterior fontanel will be found to be wider than normal and areas of softening may be detected behind the ears.

The advanced case is so striking in appearance it may be identified at a glance. The head is squarish in shape due to excess osteoid tissue forming beneath the periosteum of the skull. The rosary pattern is often exaggerated by depression or extension of the sternum and the formation of Harrison's groove. Spinal curvatures, deformities of the leg bones, etc., and delayed dentition are other features.

The highest incidence of the disease occurs late in the first year of life but it may occur earlier or much later. Maxwell and Wolfe have both reported series of cases of foetal rickets with characteristic lesions in both bones and teeth. The infrequency of such cases had previously led many to consider the fetus immune to rickets. Rickets also occurs, but infrequently, during puberty.

Vitamin D deficiency is a constitutional disturbance and more than the skeletal system is affected. Less attention has been paid to other manifestations of the deficiency than to the skeletal changes but they are helpful in recognizing the presence of the disease and their investigation may prove to be very informative. Rachitic children are usually fat and weak. Muscle weakness is evident in the lax abdominal muscles and probably in the constipation which so often accompanies rickets. Anemia is present in many cases but is due to other causes than vitamin D deficiency since it does not occur in experimental rickets and when it is present in human cases can be explained on other grounds.

Osteomalacia is most common during the period of life associated with childbearing and affects chiefly women who have repeatedly been pregnant or who are lactating. The earliest symptom is usually pain, especially in the back and sacral region. Weakness is associated and sometimes stiffness and contractures of the limbs. The muscular weakness is said to be pronounced in the adductor muscles of the thighs. If deformity follows it is first observed in the same parts in which pain occurs, namely the lower spine and pelvis. More than half of the recorded cases are said to have shown signs of tetany which is easily understood when the excessive demineralization which occurs in osteomalacia is appreciated. The loss of lime salts has been estimated to be as much as two thirds of the normal supply.

THE TREATMENT AND PREVENTION OF RICKETS AND RELATED DISEASES

The prophylaxis and treatment of rickets requires an adequate dietary intake of calcium, phosphorus, and vitamin D. The requirements of the first two are more definitely known than that of vitamin D. This is largely true because constitutional factors, the invisible intake of vitamin D through skin irradiation and the differences in efficiency of different vitamin preparations have tended to confuse the meaning of clinical trials.

Some years ago the Council of Pharmacy of the American Medical Association recommended one to two teaspoonfuls of cod liver oil daily for the prevention of rickets and three teaspoonfuls for the treatment of rickets. The recommendation was a wise one. However the average concentration of vitamin D in the better cod liver oil preparations has since doubled. Nevertheless the recommendation is still in line with present belief that the older recommendation was less than optimal. Hess at one time recommended 1100 International U.S.P. units daily. This is the same dosage recommended by Shelling and Hopper in their study of viosterol and approximately twice as much as various clinicians have found effective *in most cases*. It is well known, however, that premature infants and very rapidly growing ones require 2 or 3 times the usual dose.

For the treatment of rickets the level of dosage may be 1000 units to many times as much. The larger amounts induce more rapid healing.

Patients who do not respond to smaller doses *require* larger amounts. We have already referred to Albright, Butler and Bloomberg's study of vitamin resistant cases. Doses of from 150,000 to 1,500,000 units were sometimes necessary. In diseases such as renal rickets no response has been reported from any amount of vitamin D and Shelling and Hopper

considered vitamin D contraindicated since a tendency to metastatic calcification is frequently present in such patients.

Vollmer recommends, as treatment for neonatal and infantile tetany, severe rickets, rickets complicated by whooping cough, pneumonia or any chronic infection and as treatment of other cases of rickets when there is reason to believe the routine administration of vitamin D will not be carried out, the use of a single large dose of vitamin. His plan is to give 600,000 units by mouth. The dose is mixed with one or more feedings. Amounts less than 200,000 units do not regularly produce a favorable response but doses between 200,000 and 400,000 units are usually sufficiently large for young infants. Nádrai gave similar amounts but administered them intramuscularly.

The use of massive doses of vitamin D has generally been found safe, at any rate harmful effects have not been recognized. In the use of the vitamin in unrelated conditions such amounts may justifiably be given under supervision and without exceptional precautions. In the case of the single doses Vollmer has used, toxicity is not encountered. But, as Park points out: "In rickets the object is to restore normal calcification to the skeleton. The physician does not need to induce toxic action to bring this to pass and seeks to avoid even an approach to it." Park's recommendation is 1,200 units daily which brings the majority of cases under control within three weeks. If larger doses are required concentrated sources are used and the dose increased to the point of effectiveness. When the rachitic process has been reversed the dosage is reduced but not to the usual, low preventive level. This is necessary because 6 months is often needed for complete cure. Rachitic infants frequently manifest what may be called a rachitic constitution and this requires prolonged treatment. Jeans writes that babies who get around 400 units of vitamin D daily grow at a greater rate than those who get more. Those who get 1500 units or more "have a poorer appetite and the growth response is less."

Shohl cured [illegible] cases of rickets by administering large amounts of citrates. A mixture of 20 ml. of molar citric acid and 30 ml. of molar sodium citrate was added to the formula This corresponds to the amount of citrate in 5 or 6 large oranges. Recovery was evidenced chemically and radiographically.

The mechanical treatment of deformities is best undertaken at the earliest possible opportunity.

ATYPICAL FORMS OF VITAMIN D DEFICIENCY

The significance of subclinical vitamin D deficiency rests on observations of increased requirements of pregnant animals and the effect of

deprivation on the teeth. There are two sides to the latter problem, the effect of deficiency on the structure of developing teeth and the effect on maintaining the resistance to caries of teeth already formed.

The requirements of pregnancy and of normal tooth structure are related since rachitic lesions in the milk teeth occur before birth. For a time it was believed that the fetus was immune to rickets. This seems a rather surprising point of view since osteomalacia is commonly related to pregnancy and the combined requirements of mother and fetus are naturally greater than those of the mother alone. At any rate undeniable evidence of rickets during intrauterine life now exists, including rachitic disease of the teeth.

Maxwell and Hu reported three cases of fetal rickets and Maxwell has since collected fourteen more. In a typical instance the mother showed osteomalacic lesions of her pelvis, the infant was born with typical rachitic rosary and Harrison's groove. The anatomic evidence is thoroughly satisfactory. Wolfe has reported three similar cases and has described the lesions of the teeth. The location of the defective, hypoplastic zones of enamel and dentin were such that Wolfe could, on the basis of the established sequences in the maturation of the deciduous teeth, determine the period of intrauterine life in which the rickets occured. Histologic examination showed the predentinal zone to be widened and the dentin unevenly stained and with irregular margins. Wolfe's study is particularly instructive for it shows that deciduous teeth stigmatized by fetal rickets do not recover their normal structure with recovery from the rickets. These lesions are indelible, while the skeletal manifestations may entirely disappear.

This observation is of importance to the problem of defective teeth. Dick had found no deciduous incisors which were grossly hypoplastic and concluded that the fetus was therefore immune to rickets. But Mellanby found minute defects in 80 per cent of the deciduous teeth she examined. It is essential to her theory that either rickets may occur and disappear, leaving only dental lesions as permanent residua, or that degrees of rickets exist which are unrecognizable by our present means of examination but which are capable of interfering with the normal development of the teeth. Wolfe's studies establish that the first hypothesis may be true. That the second theory is also true is at least strongly suggested by the frequency of defective permanent teeth, formed during post natal life when the incidence of clinical rickets is a matter of record.

Infantile rickets occurs during the period when the permanent teeth are being formed, between the fourth month and the second year of life. The effects of rickets are classified according to the pattern of the lesions. Basically all are the same as they represent areas of hypoplasia. The

lesions may be in the form of wavy enamel, point or dimple defects, horizontal furrows or complete deficiency of the enamel of the crown (Freudenberg). In Mellanby's extensive examination of the teeth of British children she found only one-fifth of 1260 teeth were normal, the remainder having hypoplastic lesions of various degrees from 17.5 per cent which were slightly hypoplastic and 25.9 per cent moderately hypoplastic to 35.4 per cent very hypoplastic. The significance of this common developmental defect was not difficult to find. Ninety percent of the poorly developed teeth were also carious while ninety per cent of the normal teeth were free of caries. The ten per cent discrepancy, according to Mellanby, is due to the effect of diet subsequent to the formation of the teeth.

The association between rickets producing diets during pregnancy and rickets in the offspring has been repeatedly demonstrated in experimental studies. Hess and Blackberg in 1923 observed a varied susceptibility to rickets depending on the diet of pregnant animals. Extensive work was reported by Toverud and Guttorm who had observed a negative calcium balance during pregnancy and were able to produce the circumstances in dogs. The offspring had defective teeth. By varying the time when the bitches were fed the rachitogenic diet they were able to regulate the interval post partum when the young animals developed rickets. If the poor diet was fed throughout pregnancy the pups developed rickets three weeks after delivery. If the poor diet was fed only during the period of lactation the pups developed rickets six weeks after birth. If the diet was adequate throughout pregnancy and lactation the pups required thirteen weeks feeding of a rickets producing diet before they developed the disease. Similar results were reported by Grant and Goettsch in rat experiments in which the measure of the mother's reserves of antirachitic substance was the number of litters of rickets resistant young she could gestate.

All of these results must be interpreted in the light of the various factors involved in rickets rather than as expressions of vitamin D deficiency alone. The high calcium excretion in milk plays a prominent part and in the rat at least this effect seems only partially related to the vitamin D supply (Kletzien, Templen, Steenbock and Thomas). These authors found the calcium content of newly born rats to be fairly constant, irrespective of the vitamin D given the mother.

More specific information on the transmission of vitamin D via the placenta has recently been furnished by Toverud and Ender who assayed the livers of still born infants. Among forty-four cases about half were found devoid of vitamin D, small amounts were present in fifteen and "abundant" quantities in five. The latter group were infants whose mothers had been receiving considerable amounts of the vitamin during pregnancy. There would seem to be ample justification for the liberal use

of vitamin D and high mineral diets during pregnancy and lactation. A commission of experts has recommended 340 Int. units of vitamin D daily under such conditions.

The very nature of the rachitic lesions predisposes to the misplacement of nests of cartilage cells. This may lead to cartilaginous tumors later in life. While this is not a manifestation of subclinical rickets it forms an *interesting example of the ramifications of the deficiency diseases.* Virchow first suggested that rachitic inclusions might lead to the subsequent formation of enchondromata and numerous other pathologists have similarly interpreted certain benign cartilaginous tumors. The subject has recently been discussed by McMaster.

Vitamin D has been used empirically in various conditions not related to rickets, psoriasis, arthritis, hay fever, hemorrhage associated with jaundice, the toxemia of pregnancy and myopia. It has been used more understandably in osteogenesis imperfecta (without results—Hanson, McQuarrie and Ziegler). These reports are outside the scope of this book. It is believed, nevertheless, that other, uncommon disturbances of mineralization will eventually be recognized which may be benefited by vitamin D therapy. We have observed cases in middle aged and elderly individuals in which symptoms and lesions were suggestive of *vitamin D* deficiency.

Idiopathic and hypoparathyroid tetany can be successfully treated with vitamin D. The natural products, the fish oils, are suitable although their effect is slow. Dihydro-tachysterol, which is prepared from tachysterol and which is but slightly antirachitic has much more rapid action on blood calcium (10 times that of tachysterol). It is known commercially as "A. T. 10" and has proved to be of great value in cases where prompt action is necessary.

Chapter XXIII

VITAMIN E DEFICIENCY

Vitamin E has no proven significance in clinical medicine although it is used in various conditions on an experimental basis. Whatever the clinical uses may prove to be, if any, it requires a place in this volume both because of the striking lesions it produces in other species and because it modifies the effectiveness of the diet as regards other vitamins.

A long series of compounds have vitamin E physiologic effect to one degree or another. All are isomers or homologs of the tocopherols of which three have been isolated. They occur predominantly in plants. Animal tissues and fats contain but small amounts. Thus cod liver oil is relatively poor in vitamin E while wheat germ oil is extremely potent. They are apparently not synthesized by animals as so many vitamins are but they are so readily absorbed from the gastro-intestinal tract (per os administration is more effective than parenteral) and so ubiquitous that deficiency is difficult to imagine unless perhaps due to the instability of vitamin E in rancid oils and to ultra-violet irradiation. Excessive amounts are excreted in the feces. The normal requirements are oxidized in the body and act directly on the tissues most dependent upon vitamin E. Juhasz-Schäffer found it acted as a growth accelerator for tissue cultures. It corrects the abnormal metabolism of muscle from deficient animals when added directly to excised samples. Vitamin E requirements increase as the size and number in the litter (Evans and Burr). The small amount of stored vitamin in the tissues is principally in the liver and mammary gland.

SYNERGISTIC ACTION WITH OTHER VITAMINS

It is well known that the E vitamins exert a marked protective effect on other vitamins and on all fats. Mention has been made of the effect vitamin E exerts on vitamin A requirements. The effect of both carotene and vitamin A are doubled in the presence of tocopherol. This effect presumably takes place in the intestinal tract for if the two are administered on alternate days the protective action is much reduced (Hickman). It has also been demonstrated that small addenda of tocopherol increase the storage of vitamin C in the guinea pig. The addition of 0.5 to 1 mgm. tocopherol per kg. body weight increased the liver ascorbic acid roughly 20 per cent and the adrenal store 20 to 60 per cent. On the other hand Harris found that tocopherol reduced the antirachitic action of small

doses of vitamin D. Vitamin E also enhances the effect of progesterone by 50 per cent and to a slight degree the action of estrogen (Stähler and Pehl).

The relationship between vitamin E and anthracenes suggested to Adamstone that the vitamin may be necessary to the utilization of compounds of this kind. Thus the association of brain cholesterol, testicular androgen and uterine estrogen with the characteristic lesions might readily be explained. Adamstone planned three experiments to test his hypothesis. He attempted to correlate the cholesterol content of the chick brain with the development of lesions and found a time relationship. He administered testosterone to groups of caponized males and found that there was little response in birds on a vitamin E deficient diet. And finally he showed that vitamin D effect in the liver was modified by the vitamin E value of the diet.

While the morbid effects of vitamin E deficiency vary a great deal between different species, the differences are not fundamental and probably will some day be shown to have a single pathogenesis. The vitamin appears essential to certain vital functions associated with rapid cellular multiplication rather than to a particular tissue, as is true of vitamins C and A. Juhasz-Schäffer and Mason, both on the basis of extended study of the histologic effects of vitamin E deficiency, associate the vitamin with cellular division.

Mason considers that the nucleus is affected because the damage is irreversible, suggesting a more central and fundamental damage than would be the case if the cytoplasm alone were affected and because the first visual evidence of the deficiency is in the structure of the nucleus.

This theory would seem to explain the major effects of vitamin E deficiency. The application to the testis and fetus is obvious. In both case cellular division is extremely rapid. The nervous lesions are not dissimila The paralysis is closely associated with the weaning period which is als the time of greatest nuclear activity in the cerebral cortex of the rat. similar opinion was reached by Adamstone and Card from histologic stu of the testes of fowl. Adamstone has also followed the sequences in t egg embryo and ascribes fetal death to damage to the blastoderm, vessels of which are destroyed and cause the death of the embryo starvation and/or anoxemia.

VITAMIN E DEFICIENCY IN THE RAT

The effects of vitamin E deficiency have been determined main experiments on rats. The functional result observed is the product sterility in both sexes and hence the name 'anti-sterility' vitamin. term is to a degree misleading, for other pathological results occur

PLATE XLV. Effect of vitamin E deficiency in rat foetus, showing petechiae a[illegible] venous ectasia. (Courtesy of Dr. Karl E. Mason.)

are in no way related to fertility. It seems [illegible] tion of vitamin E and sterility is due to the [illegible] the fetus and testis and that other tissues also require the [illegible] their period of rapid growth.

The functional effects of vitamin E deficiency in the male [illegible] four months' depletion and develop through four stages. The first stage consists of a period when normal sex responses are present as well as normal appearing sperm cells but the sperm lacks the power to [illegible] ova. Later, sperm cells are entirely lacking from the [illegible] loss of power to form the bouchon appears and finally the [illegible] interest as well.

The germinal tissue of the female is not affected and [illegible] follicles appear perfectly natural. These tissues are [illegible] A depleted female conceives normally but the fetuses [illegible]

Placental tissue, implantation site, and fetal tissues are all affected in vitamin E deficiency. The implantation site is [illegible] by the presence of many free erythrocytes and hemorrhages [illegible] in the placenta. The sinusoids are tremendously distended. The fetal [illegible] are predominantly in the hematopoietic and [illegible] The depressed hematopoietic activity was originally believed to be the cause of the fetal deaths but Mason has since emphasized the importance of the vascular lesions. Based on a tremendous number of [illegible] an abbreviated bio-assay technique, Mason's theory is that the vascular lesions not only account for fetal death but possibly explain the encephalomalacia seen in chicks and the exudative diathesis described by Dam and Glavind. Many viable fetuses, examined during the 16th day of gestation, are normal but others are pale with scattered areas in which the blood vessels are distended with stagnant blood.

The superficial vessels are first affected in the following regions, cranium, external ear, shoulder and dorsolateral portions of the trunk. Capillary petechiae may be found in these areas and plexiform dilatations of the venous channels. The large venous trunks in the neck were frequently distended and the site of large extravasations of blood in the walls and those of the cerebral vesicles. It is especially interesting to note that Mason found these lesions in fetuses in which normal mitosis was still present in other organs. Thus through asphyxia or otherwise the products of conception die and are slowly resorbed. (See Plate XLV.)

The third functional effect of vitamin E deficiency is seen in the offspring of depleted mothers. Under such circumstances and at about three weeks post partum a paralysis develops which resembles [illegible] neurone lesion. This [illegible] ticular moth[illegible]

vitamin A and E deficiencies is due to the frequent combination of the two and if adequate vitamin E is supplied, the testes from the A deficient animals may be readily distinguished from those of E deficiency.

In A deficiency the germinal cells slough off at an abnormal rate and the denuded seminiferous tubules gradually shrink in size. Some maturation continues in the deeper layers of the germinal cells, probably at a greatly reduced rate. One other typical result of vitamin A deficiency is said to be the persistence of nests of maturing cells. However, in prolonged and severe deficiency a stage of atrophy is reached which is indistinguishable from the fifth stage of vitamin E deficiency.

In common with most other avitaminoses the lesions of vitamin A deficiency are rapidly reversible and in experimental studies this serves as a valuable differentiation between these less specific effects and the results of a deficiency of vitamin E. In inanition, as in other vitamin deficiencies, sloughing of the germinal epithelium is less pronounced than in A deficiency. The end stage of the atrophy of inanition resembles the uncompleted seminiferous tubules seen in immature animals.

While vitamin A and E effect the testis in different fashion, Mason has found a relationship between the action of the two. The lesions of vitamin E deficiency may be retarded if vitamin A is also lacking. Mason's explanation of this phenomenon is that the maturation rate is retarded by the deficiency and less vitamin E is needed to insure natural nuclear function and structure.

Associated with the permanent sterility of prolonged depletion are lesions of the uterus and ovaries. The former become yellow-brown in color (brown atrophy) and the ovaries contain large corpora lutea. Patchy areas of fatty degeneration may be found in the musculature. Virgin rats develop these lesions as well as animals which have undergone a pathological pregnancy with resorption. Vaginal bleeding and fibromyomata are sometimes seen (Barrie).

MUSCLE DYSTROPHY AND VITAMIN E

An early observation of vitamin E deficiency was the presence of paralysis in the suckling young of depleted rats. Evans and Burr found this to be a regular manifestation before the 25th day of life. In some an initial paretic stage was seen but in most spasticity was the first sign. The spasticity was limited to the hind quarters and eventually was associated with symmetric baldness and muscular atrophy. Early treatment with vitamin E was sometimes curative.

Similar lesions can be produced in adult rats (Ringsted) but require [illegible] months or longer of a deficient diet. The symptoms commence with dragging of the legs, slight incoördination and some thinning of the hair. The

adductor muscles weaken and the gait becomes straddling in type. Finally the hind quarters are dragged about and cannot be adducted.

VITAMIN E DEFICIENCY IN HERBIVORA

More extensive but otherwise indistinguishable lesions occur in herbivora after relatively short feeding periods. They were first described by Goettsch and Pappenheimer in guinea pigs and rabbits. Weakness progressing to complete helplessness and associated with extensive hyaline necrosis of the striated muscle rapidly develops. The lesions are thought to be extraneural since the terminal nerve structures are intact but section of the sciatic was found by Pappenheimer and Goettsch to afford complete protection.

The lesions were first induced by feeding natural foods treated with iron and there was some doubt that vitamin E was the sole causative factor. Additions of cod liver oil also predisposed to lesions and Madsen, McCay and Maynard suspected that a toxic substance in the oil played a large part. Morgulis and his colleagues demonstrated two factors were involved, vitamin E and a water soluble constituent in yeast and other B sources. The harmful effect of cod liver oil is limited to the herbivora, rats and mice utilize ingested vitamin E despite the presence of it. The likelihood that other species may have their vitamin E requirements jeapordized by feeding large amounts of fish oils has been suggested by several isolated observations and deserves attention.

Herbivora lose weight and cease growing during vitamin E deficiency in contrast to rats which maintain a normal appearance. Preceding the muscle lesions rabbits demonstrate decreased muscle creatine, creatinuria and an altered creatine-creatinine ratio in the urine.

Verzar reports that creatinuria is regularly associated with muscle dystrophy in rats and responds very rapidly to treatment with α-tocopherol. In a single day the excretion of creatine decreased three-fourths and the ratio of creatine to creatinine from 40 to 130 per cent to about 5 per cent. The water and chloride content of the muscle is increased. Knowlton, Hines and Brinkhous followed these values as well as the creatine content and found water and chloride values to increase before weakness was manifest. Synthetic α-tocopherol controlled all of these signs.

Many tests have been made on living and dead muscle from E deficient animals, largely by Matill, Houchin and associates. In Warburg experiments it was shown that the dystrophic muscle exhibits an increased oxygen uptake. In improved experiments using hamster muscle Houchin found the increase to be 250 per cent and to return to near normal within 27 hours after vitamin E was administered. A 34 per cent decrease was noted within the hour. The same response could be demonstrated by

adding α-tocopherol phosphate directly to the excised sample. Kaunitz and Pappenheimer, in confirming many of these results, also demonstrate that the chemical lesion precedes the anatomic one.

The lesions in mice are intermediate between the rat and the chick (to be described later). The testis remains normal both in structure and fertility of the sperm. Hyaline necrosis of the muscle occurs but infrequently and edema of the subcutaneous tissues and muscle stroma is relatively common (Pappenheimer). No lesions have been observed in the central nervous system.

The guinea pig is relatively immune to testicular degeneration. The early stages require 4 to 5 months to develop, twice as long as is required by rats. Muscular dystrophy appears much earlier.

As has been mentioned the muscle lesions were originally observed only in young animals. Mackenzie, Mackenzie and McCollum succeeded in producing identical lesions in rats which had been on a normal diet until full grown. The time necessary for the production of the dystrophy was 8 to 10 months. The first symptom was spreading of the hind legs and lowered posterior abdomen when walking. After 45 to 50 weeks extreme abduction of the hind legs was seen and finally the hind legs became lifeless. Tremors and incoördination of the forelegs and head were noted shortly afterward and became so severe they interfered with eating. Excitement and noises intensified the tremors and even induced convulsions and coma. The muscle lesions were characteristic. Therapy was without effect.

Thus the two important factors in vitamin E deficiency production are duration of the depletion and body store of vitamin at the beginning of the experiment. Since depletion is slow and the requirements not large great variations are possible. Mason and Bryan have discussed this as it applies to the biological assay of vitamin E sources. A deficient diet during the last half of lactation minimizes the reserves of the off-spring. Testicular degeneration can be hastened by this means and lesions occur coincidently with the first appearance of spermatozoa.

Daft, Endicott, Ashburn and Sebrell have found that the addition of succinyl sulfathiazole to a purified diet which was adequate for rats was followed by the development of hyaline necrosis of various voluntary muscles. Alpha-tocopherol was preventive. This is, therefore, an alternative method for hastening vitamin E deficiency.

Vitamin E deficiency results in morphological changes in the anterior lobe of the hypophysis where enlarged, vacuolated basophilic cells (castration cells) appear according to van Wagenen. Verzar suggests that the retarded growth, lowered basal metabolism and inferior fur of deficient rats are due to malfunction of the hypophysis and that the vitamin may

be necessary to the formation of the hormone. These and
deficiency cannot be explained on the theory of retarded nuc

VITAMIN E DEFICIENCY IN DOMESTIC FOWL

In 1931 Pappenheimer and Goettsch observed a striking dis they suspected was due to a nutritional disturbance among ch simplified diet. Some years later it was recognized in the field by It is known in New England as Crazy Chick Disease. Subsequen established that it is due to a deficiency of a factor in various edi later identified as vitamin E (Dam, Glavind, Bernth and Hagens).

The symptoms develop suddenly after a period of rather good g and normal behavior. Ataxia, coarse tremors, retraction of the opisthotonus, forced movements, clonic spasms, prostration and deat the common sequences. If undisturbed the chicks droop and remain long periods with their eyes closed. Complete paralysis does not oc Some birds have mild symptoms and recover and in rare cases extens lesions develop without clinical manifestations. The peak incidence occu during the 3rd and 4th weeks of life. Older chicks are less often affecte but the disease develops more rapidly when it does occur.

The characteristic lesions occur in the brain and most commonly in the cerebellum. The cerebrum, medulla and mid-brain may be affected in that order. The cerebellum is swollen, soft, and stippled with minute hemorrhages. As necrosis proceeds the lesions assume a greenish yellow, opaque appearance. As much as $\frac{3}{4}$ of the cerebellum may be diseased. The histologic characteristics are edema, degeneration and necrosis of the Purkinje cells and the small cells of the granular layer, small hemorrhages and hyaline capillary thrombi. The thrombi are the immediate cause of the necrosis and a very constant feature of the lesion. The cerebellar location is believed to be determined by its more indirect vascular connections. During healing the capillary endothelium becomes hyperplastic.

The spinal cord and peripheral nerves are unaffected. A few chicks show subcutaneous edema.

The spontaneous disease has been reported from most poultry diagnostic laboratories in New England and at the Storrs Experiment Station accounts for nearly 8 per cent of all chicks between 2 and 8 weeks of age submitted for diagnosis. It is the 5th most common chick disease in that age group.

The simplified diets which cause "nutritional encephalomalacia" in chicks causes an almost universal degeneration of the skeletal muscles in ducklings and a selective necrosis of the smooth muscle of the gizzard in young turkeys. This species difference is one of the most remarkable aspects of vitamin E deficiency in birds.

PLATE XLVII Encephalomalacia in the chick The upper drawing is of a normal (left) and diseased (right) animal and shows the extensive lesions sometimes seen in the vascular system the contents of which were selectively stained

The lower drawing is of an area of cerebellar softening Both drawings were made of illustrations prepared by Pappenheimer, Goettsch and Jungherr (Courtesy of Dr. A M Pappenheimer)

Ducklings, during the 2nd and 3rd week of life, become extremely weak and sprawl or stagger. They often become too weak to lift their heads. Lesions are found only in the skeletal muscles which are very pale and on microscopic examination found to be destroyed by waxy or hyaline degeneration. The creatine content of the muscles is greatly reduced, the conditions being similar therefore to vitamin E deficiency in rabbits. This disorder also occurs spontaneously (Seifried and Heidegger) but under natural conditions is associated with necrosis of the gizzard muscle.

Necrosis of the gizzard muscles was first produced experimentally by Jungherr and Pappenheimer in 1937 by feeding the same restricted diet used in chicks to young turkeys. No symptoms were noted but greyish patches were seen in the gizzard and found due to hyaline necrosis and reparative phenomena in the smooth muscle coats. This lesion but rarely occurs spontaneously.

Adamstone has described lesions in the chick embryo which he associated with vitamin E deficiency. He has found them both in experimental and natural circumstances. The lesion is a focus of hemorrhage marked by a rosette of cells with pyknotic nuclei and clear cytoplasm. Similar rosettes were found unassociated with hemorrhages. Adamstone believes that many chick embryos which die during the first week of development may represent instances of vitamin E deficiency.

These observations may possibly be correlated with those of Dam and Glavind and Dam who described an exudative diathesis in chicks deficient in vitamin E. Grossly large accumulations of fluid are found in the subcutaneous tissue and muscles. The lesions are diffusely hemorrhagic, the extravasated fluid of a pale green color. Many of Dam and Glavind's birds spontaneously recovered for some time only to die of perforating erosions of the gizzard. An abnormal gradient for colloidal dyes was demonstrated to be associated with the exudative diathesis. In extensive studies by Dam it was shown that both the exudative diathesis and encephalomalacia could be exaggerated or suppressed by various alterations of the diet other than in its vitamin E content. Certain fatty acids produced exudation, others encephalomalacia. Inositol counteracted both lesions, lipocaic only the exudative diathesis.

With a slightly different diet Bird and Culton produced hydrothorax and hydropericardium as well as a generalized exudation into the soft tissues.

Adamstone also reports swelling and degeneration of the livers of E deficient chicks. Scattered areas of a mahogany color were found to be the site of extreme phagocytosis of erythrocytes by the Kupffer cells and the formation of hemosiderin. The marrow was hyperplastic. He attrib-

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CHAPTER XXIV

VITAMIN K DEFICIENCY

Certain vitamins are important chiefly because of the part they play in nutrition. Others are important nutritionally and pathogenically. Vitamin K seems to be important solely as a pathogenic factor. What contribution it makes to nutrition is unknown.

Vitamin K (more properly vitamins K) is synthesized in plants and by certain microörganisms including many common inhabitants of the intestinal canal. Persistent and severe diarrheas therefore reduce the endogenous supply. Vitamin K is fat soluble and absorbed only in the presence of bile salts, especially desoxycholic acid. Thus obstructive jaundice interferes with absorption not only of the dietary but also the synthesized supply and more severe deficiencies result from jaundice than from diarrhea. And finally, since vitamin K can function only in cooperation with a normal liver, all forms of organic liver disease may interfere with the proper use of what vitamin K is ingested, synthesized and absorbed.

The important rôle of vitamin K is in prothrombin formation. Prothrombin is apparently formed in the liver and largely destroyed in the lungs. The concentration of prothrombin represents a condition of balance between formation and destruction. Vitamin K is essential in prothrombin formation, although it does not enter into the prothrombin molecule. It has no effect on the upper limits of concentration. That is excess vitamin K does not increase the prothrombin concentration above a certain level (Quick).

EXPERIMENTAL VITAMIN K DEFICIENCY

Dam's original experiments, which were devoted to sterol metabolism, simply mentioned the hemorrhage and weakness observed in certain chicks and that orange juice did not correct the disorder. More information was given in a later report (Dam and Schønheyder). Hemorrhages had been noted in 60 to 70 per cent of the chicks reared on the basal diet. The sign appeared after 11 days of depletion, in most cases between the 15th and 20th days. The location of the hemorrhages was determined largely by trauma. Thus the left breast was the common site because injections were made there. Hemorrhage could be induced by pinching. The hemorrhages were predominantly subcutaneous and of various sizes. Intramuscular hemorrhages were very common in the legs and often the wings were marked by extravasations. There was no predilection for the knee

joint although in a few cases blood was found within the joint. Subperiosteal hematomata were never seen. In a few animals considerable amounts of blood were found in the peritoneal cavity and petechiae were seen at times in the liver. Some chicks were edematous, one had a retrobulbar hematoma.

Brownish areas of a frayed or ulcerated appearance were found in the gizzard. The pigmentation was due to blood. These lesions varied in size from minute spots to areas 1 cm. in diameter. In some cases tarry material was found in the gizzards. In a few chicks patches of hyperplastic epithelium were seen in the cardia. The gizzard lesions consisted of congestion, hemorrhagic infiltrations and superficial ulcerations. Atrophic changes occurred in the gastric glands, the columnar cells becoming shortened, the gland fundi dilated.

McFarlane, Graham and Richardson described their experience with vitamin K deficient chicks as follows The mortality rate was very high, half of the deaths being due to hemorrhage following the insertion of identification bands into the wings. Bleeding from the small wounds so made continued for from 12 to 24 hours, the feathers being continuously wet with blood. In the remaining chicks large hematomata were found beneath the skin along the femur, particularly the left, the ribs and pectoral muscles. Blood from such animals failed to clot on standing overnight.

Dam, Schønheyder and Lewis had little success in producing vitamin K deficiency in laboratory animals. Hawkins and Brinkhous demonstrated that deficiency could be induced by establishing a biliary fistula and similar results were secured in rats by Greaves and Schmidt. In these animals as in the chick the important lesion was a failure of the blood to clot apparently due to diminished prothrombin. A very satisfactory technique for establishing deficiency in the rat has been devised by Flynn and Warner and consists of placing rats on a diet low in vitamin K from the time of weaning and later ligating the common bile duct. A degree of deficiency can be induced by dietary means alone but never profound enough to depress the prothrombin value greatly. A severe depletion of vitamin K requires deficient intake plus biliary obstruction. Under these circumstances the prothrombin concentration falls slowly during the first 24 hours and thereafter rapidly until after 3 days the values are characteristically 10 to 25 per cent of normal. At the lower value rats invariably bleed unduly from venepuncture or scratches, at 25 per cent they frequently do. Biliary occlusion alone is more effective than simple depletion in establishing a severe prothrombin deficiency but even after 3 weeks of biliary obstruction the values range between 20 to 40 per cent and thereafter biliary cirrhosis develops and the animals are no longer suitable for most experimental studies. It is interesting to note that under conditions of depletion

death were: lobar pneumonia, 5; hemorrhagic colitis, 3; erysipelas-like infection, 1; cause not established, 4.

Many inquiries have been made of the influence of ascorbic acid on the course of tuberculosis. This has been due, no doubt, to the realization of the large part diet plays in the control of clinical tuberculosis, a phenomenon which has never been satisfactorily explained. Tuberculosis and scurvy have a common history. If one investigates the epidemics of scurvy one is constantly reminded of the simultaneous frequency of tuberculosis. Aschoff and Koch's large series of necropsies of scorbutics revealed that tuberculosis was the common cause of death. Of those due to other diseases most were the result of other infectious diseases.

Scurvy modifies the anatomical responses to tuberculosis in at least two ways, by predisposing to hemorrhage and by preventing the evolution of a proper fibrous tissue scar. Robert Koch said that scurvy was a serious hazard to tuberculous individuals because of the tendency to hemorrhage. The point of inoculation of tubercle bacilli frequently becomes hemorrhagic and even the lesions in the spleen and other organs are surrounded by extravasated blood.

Höjer extensively studied the histopathology of experimental tuberculosis as it is influenced by diet. Two of his illustrations are particularly illuminating. They contrast the collagen poor, irregular scar about a tuberculous focus in an animal moderately depleted of vitamin C and the solid, compact scar in a well fed pig. This appears to be a thoroughly reliable observation which harmonizes with what has already been said of the influence of ascorbic acid on the formation of collagen. The same mechanism probably explains those cases seen by Aschoff and Koch in which old tuberculous lesions were activated and an acute, exudative inflammation spread from them. Mazoué's work is confirmatory. Wolbach has written: "In diseases, notably the infectious granulomata, where fibrosis of lesions is the important element in arrestment of progress, avoidance of C deficiency is of supreme importance." It may be necessary to broaden this statement. Wachsmuth and Heinrich reported a case of scarlet fever complicated by starvation. Four months later bone pain led to an exploratory operation. A sequestrum, infected with streptococci, was found. In commenting on this case Takahashi ascribed this metastasis of the infection to the nutritional condition (probably scurvy) and said that in his own experiments scurvy had produced foci of low resistance so that pathogenic microörganisms injected intravenously induced disseminated lesions in all scorbutic guinea pigs but in less than half of the controls.

The sensitivity to tuberculin has been investigated. Recent studies by Birkhaug illustrate the general outcome of such studies. Birkhaug tested

two groups of 12 guinea pigs each for sensitivity to tuberculin on the 24th, 51st and 62nd days following injection of a uniform dose of bovine tubercle bacilli. Both groups were fed an "adequate" diet but one received a supplement of 10 mgm. ascorbic acid daily. Chemical tests of urine and adrenal tissue revealed that the control group became slightly depleted of vitamin, the experimental animals remained saturated. They were likewise less sensitive to tuberculin. Dissection and histologic studies confirmed Höjer's results. The ascorbic acid poor pigs showed more caseous lesions, less collagenous scar and more dissemination of the infection. Heise, Martin and Schwartz have conducted similar studies in human cases. The administration of ascorbic acid supplements was shown to reduce the skin sensitivity to tuberculin. The 24 hour erythema readings in the controls averaged 351 sq. mm as compared to 76 sq. mm. in the patients given ascorbic acid. In another of a series of articles on the subject Heise and Martin reported a failure to demonstrate any clinical benefits in tuberculosis from massive doses of ascorbic acid.

Comprehensive experiments and clinical investigations have been reported by Steinbach and Steinbach and Klein. In a discussion of the phenomena in experimental (guinea pig) scurvy they state that ascorbic acid treatment over a considerable period leads to a heightened resistance to tuberculin. They were able to establish conditions under which the treated guinea pigs survived doses of tuberculin which regularly killed control animals. The adrenal glands were examined for ascorbic acid (silver nitrate test) and it was discovered that the cortex was depleted of vitamin in those animals which had been killed by tuberculin.

We have ourselves observed among pigs injected with routine sputum concentrates and urine that animals on an inadequate diet develop more extensive lesions and are more susceptible to tuberculin shocking than pigs adequately fed.

Clinicians have generally reported benefits from vitamin C rich diets but in most of these reports the differences have not been as striking as those in experimental tuberculosis or, it seems proper to suggest, as they would be under circumstances in which the basal diet was deficient. Clinical studies are in agreement in another respect. Patients suffering from tuberculosis are almost uniformly found to have abnormally low blood values of ascorbic acid, to be partially depleted. The same is true for most infectious diseases. On the other hand capillary fragility, another sign of scurvy, is seldom present in clinical tuberculosis. An exception appears to be the patients with hemoptysis. Borsalino investigated such cases. Capillary fragility was frequently present in cases with hemoptysis and was amenable to treatment with ascorbic acid. Hemoptysis ceased as the fragility disappeared and recurred when treatment was discontinued.

In the first edition of this book we discussed the studies of Rineha who observed changes in experimental scurvy which were suggestive those in rheumatic fever. Rheumatic fever is a disease of the poor a this and other characteristics of it suggested a nutritional relationship a led Rinehart to consider mild forms of scurvy worthy of further stud Many investigators have explored the problem since then. Rinehart observations have been confirmed but his interpretation has been the su ject of much disagreement. Disagreement has centered on two point The lesions have not appeared to all pathologists to be characteristic rheumatic fever and the theory that subclinical scurvy has etiological si nificance has been denied on the basis of clinical trials in which even co siderable amounts of the vitamin have failed to prevent recurrences. Th blood of rheumatic fever subjects is usually depleted of ascorbic acid, in the case of tuberculosis, but that is true of infectious diseases as a clas However, not many infectious diseases seem to influence the blood level as greatly as rheumatic fever does. Rinehart reports that even cases rheumatoid arthritis, in 93 per cent of his cases, had blood values belo 0.5 mgm. per cent. Rheumatic fever patients usually have capillar fragility as well as low blood levels of vitamin.

Several observations have been made in the course of these studies whic are of considerable interest. Taylor believes that scurvy produces a car ditis without superimposed infection; that if allowed to persist it reaches stage of irreversibility as far as ascorbic acid influence goes. Valvulitis myocarditis, pericarditis and arthritis all occurred in his animals and the carditis was capable of causing congestive heart failure. The lesions fre quently contained microörganisms although none were injected indicating that a latent infection had been activated by the scurvy. In Rinehart and Schultz' experiments, infection was induced by injecting streptococci. The present status of this problem would seem to be that definite evidence of the part played by ascorbic acid in the etiology of rheumatic fever is lacking but that the synergistic effect of mild scurvy and infection has been confirmed in critical and extensive experiments.

The most recent addition to these studies has been made by Schultz and Schultz and Rose. Schultz sought to explain the findings of Rinehart on the basis of increased metabolism. Both thyroxin and insulin were found capable of producing the type of carditis associated with scurvy. Whether this will be the final explanation of the influence of vitamin C or not remains to be seen. Evidently the action is not a specific one.

The effect of vitamin C on toxins has been the subject of many reports. Harde was a pioneer in this field. She felt a considerable similarity existed between the lesions of scurvy (in the adrenal) and those due to the injection of diphtheria toxin and that animals, like the mouse, which synthesize

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vitamin C were resistant to certain toxins because of this ability to synthesize ascorbic acid while guinea pigs which are susceptible to the toxins are susceptible because they are dependent on dietary vitamin. These observations led to experiments which seemed to demonstrate that ascorbic acid was capable of both an in vitro and in vivo inactivation of diphtheria toxin. King and Menten and Jungeblut and Zwemer reported similar results and associated the effect of the toxin on the adrenal with its ascorbic acid content. Pakter and Schick have reviewed these and other experiments and ascribe the effect to the reducing property of ascorbic acid and in certain experiments to the pH of the mixtures used. They failed to correlate vitamin C with the Schick reaction in children. The experimental evidence is quite contrary but seems to demonstrate a direct effect. Torrance's experiments, for example, clearly show an inverse correlation between adrenal hemorrhage and vitamin C content and that additional vitamin C reduces both the hemorrhagic response to diphtheria and mortality. Torrance speaks of the adrenal changes as a "localized scurvy." Torrance found meningococcus filtrates capable of the same effect on the adrenals and their vitamin content. Frequent clinical reports speak of the benefits to be derived from treating diphtheria with ascorbic acid and antitoxin. Kumagai wrote that 400 to 600 mgm. daily reduced the death rate among cases of severe, necrotizing diphtheria. The subject of ascorbic acid and toxins must be considered as open

Jungeblut extended his observations of the effect of vitamin C on diphtheria toxin by studies of the action on the virus of poliomyelitis and on the experimental disease The virus was rapidly inactivated in vitro. Animal experiments seemed to show that doses of ascorbic acid at a definite level gave protection against the disease. However Sabin was unable to confirm this. In a discussion of the relationships between ascorbic acid and poliomyelitis Heaslip reported observations made in Australia. He had noted the frequent reference in the literature to the common susceptibility to diphtheria and poliomyelitis. In 1917 Zingher found that a disproportionately high percentage of poliomyelitis cases in New York City occurred among Schick positive children. This was also true of the Australian cases. Heaslip also studied the urinary excretion of vitamin C and showed that a further characteristic of such children was a low excretion of ascorbic acid, that is a form of depletion.

These and similar studies have brought sharply to our attention the significance of different levels of vitamin C nutrition. Dietary habits are doubtless of the greatest importance in determining this characteristic but individual factors are also important. It seems justified to ask whether these are all but expresssions of an underlying constitutional character rather than the reflection of eating habits.

In the matter of vitamin C metabolism during febrile diseases it has been learned that most, if not all, febrile diseases do lower the vitamin C levels in blood and tissues. Frequently this depletion deepens to a stage of significance to the recovery of the patient. The action is not understood. Daum, Boyd and Paul and Zook and Sharpless have shown that artificial hyperpyrexia causes a fall in blood vitamin. Possibly fever alone is responsible. The intake of ascorbic necessary to maintain "normal" level in the blood plasma is often quite large during infectious diseases. Faulkner and Taylor describe a case of tuberculosis in which 200 mgm. per day were needed. We have followed patients who required still more.

One further aspect of this problem seems important. A study of the symptoms of many cases of prolonged infectious diseases reveals many lesions which may be due in part or entirely to secondary vitamin deficiency. The Zenker's degeneration of the abdominal muscles and excessive bleeding in typhoid fever may be better explained by a superimposed scurvy than by the typhoid itself. By high caloric diets Coleman radically improved the prognosis and shortened the duration of typhoid fever. It is reasonable to believe that further improvement could now be made by providing an adequate vitamin intake.

A comprehensive study of the effect of vitamins on one factor in resistance to disease, specific antibody production, has been made by Jusatz. Rabbits were used. They were fed autoclaved diets which caused a form of ill health. To this were added various vitamins and the various groups were then immunized to horse serum. The criterion used was the formation of precipitins. Oral administration of vitamins A, B complex, D and C was without effect but the subcutaneous injection of large amounts of ascorbic acid trebled the titer of the serum. Jusatz also found that the ultimate titer of the serum was increased by intravenously administered ascorbic acid, injected immediately preceding the injection of antigen.

VITAMIN A

Some years ago vitamin A was frequently spoken of as the "anti-infectious" vitamin. This was due to early studies of rat avitaminosis. McCarrison and Mellanby both emphasized the frequent association of inflammatory lesions. Mellanby, for example, reported that among 92 rats on an A deficient diet 44 per cent had urinary tract infections, 20 per cent had otitis media, 21 per cent enteritis, 9 per cent pneumonia and 95 per cent abscesses in the floor of the mouth. For a time indeed it was believed that infection preceded the epithelial metaplasia and produced it.

Later work disproved this view. It is now generally conceded that the metaplasia is the primary effect of deficiency, that it is of such a nature that the impenetrability of the epithelium is destroyed and that microör-

ganisms are thus enabled to invade the epithelial surfaces which are affected. Vitamin A is "anti-infectious" in the sense that it preserves the normal cellular barriers against infection. The effect may not be entirely structural. Thus Sullivan and Manville found changes in the lysozyme production of the intestinal epithelium. Lysozyme is the name given the bactericidal factor discovered by Fleming in many body fluids. Findlay, in 1925, and Anderson, in 1932, had shown that its production is diminished in vitamin A deficiency. In 1937 Prickett, Miller and McDonald reported it was increased. Sullivan and Manville were able to explain this discrepancy. They found that while the content of the bowel wall was increased the secreted lysozyme was greatly reduced in amount. (See biotin lysozyme relationship, Chapter XX.)

The indispensability of vitamin A to epithelial structures suggest that these phenomena may be characteristics of this deficiency alone. Possibly the same mechanism is responsible for the many claims that vitamin A is preventive of common colds Cod liver oil and concentrates of cod liver oil have enjoyed a widespread popularity as cold preventives. Beard reported that the severity of colds was reduced among 36 students who had taken cod liver oil steadily throughout a year. Holmes and his associates, working with an industrial group, reported a reduced incidence of infection and 40 per cent less loss of time among employees given cod liver oil. Shibley and Spies, on the basis of an extensive experiment, concluded that vitamin A had no effect on the incidence or severity of colds although there was suggestive evidence that the duration of the illness was shortened. Gardner and Gardner found that the severity and duration of colds was reduced in a group given supplements but no reduction in the average number of infections

The common cold is a complex infection in the sense that it is inaugurated by a virus and that the prolongation of the illness and the later symptoms are due to secondary bacterial infection. This may explain the results just summarized. Thus the respiratory epithelium could conceivably be no more resistant to invasion by the primary pathogen, the virus, and yet be more resistant to the secondary, bacterial infection. Under such circumstances the incidence of colds would not be reduced but their duration and severity would be

At the present this is purely speculative since there is no agreement that the course of the common cold is definitely influenced by vitamin A supplements. The problem cannot be solved until much more is known of intake and requirements. Certain observers have had favorable results and others unfavorable because the nutritional status of their patients was quite different. Thus Hess and Barenberg found that vitamin A did not prevent or reduce the incidence of the common infectious diseases among the infants

in their institution but since dietary problems had been earnestly studied there for many years we may assume that the basal diet, to which supplements were added, was fully adequate to begin with.

Some experimenters have attempted to measure the relationship of vitamin A and resistance more accurately. Clausen's experiments require attention on this account. He correlated dietary supply of vitamin and plasma carotene content with resistance to infection. Infectious diseases were more common among infants 6 to 24 months old who had not received cod liver oil or vegetables and whose plasma carotene was low. Scarlet fever was less severe in children whose plasma carotene was of a high value at the beginning of the illness. Plasma carotene fell during infectious diseases, especially during the more prolonged illnesses. This was due in large part to the reduced food intake. Clausen obtained certain anomalous results which cannot be explained at present but his experiments point the way to better study of such problems.

Various attempts have been made to determine whether vitamin A has value after the infectious agent has passed the epithelial barrier. Boynton and Bradford demonstrated that A deficient rats were less resistant to certain virulent microörganisms injected intraperitoneally. Smith and Hendrick reported that deficiency reduced the resistance to tuberculosis. Lassen extensively studied the course of salmonella infections in deficient rats. The deficiency predisposed to generalization of the infection. McClung and Winters had similar results. Repeated passage through deficient animals did not increase virulence, however. Lassen demonstrated some inferiority in the formation of antibodies. His monograph and that of Robertson may be recommended for a review of the literature.

During vitamin A deficiency the Kupffer cells become swollen and degenerate. It is quite possible that a part of the effect of vitamin A deficiency on infectious diseases is due to this involvement of the reticulo-endothelial system.

In most experiments in which an increased susceptibility has been clearly shown the degree of deficiency has been considerable. Thus Lassen found the stage of diminished resistance present only when xerophthalmia was evident. Comparable degrees of deficiency are not common among our sick.

The stigmata of vitamin A deficiency are relatively permanent as has been mentioned in an earlier chapter. Sherman and MacLeod found that deficiency during youth conditioned the resistance throughout life. Thus in rats "there appeared in early adult life an increased susceptibility to infection, and particularly a tendency to break down with lung disease at an age corresponding to that at which pulmonary tuberculosis so often develops in young men and women."

VITAMIN B COMPLEX

In the early studies of vitamin B and infectious diseases the B complex was widely used and it is impossible to assign the results to particular components of the complex. Perla's review may be consulted for work published prior to 1938. The most extensive work was reported by Kuczynski who used various diets of natural foodstuffs and tested the resistance of mice to intracerebrally injected yellow fever virus. B_1 deficiency was most effective in reducing resistance.

Leprosy has frequently been used as an experimental disease because clinicians familiar with it have observed the importance of diet. Thus Lamb demonstrated that diets low in B and calcium reduced the time required for the evolution of leprosy nodules in rats and caused more extensive lesions He extended his studies over several generations of animals on a slightly deficient diet and discovered that the 3d and 4th generations showed an increased susceptibility. These studies have been extended by

TABLE XXXVIII

EXPERIMENT NUMBER	DIET	NUMBER OF RATS	PER CENT OF RATS HAVING PALPABLE LEPROMATA AT VARIOUS WEEKS					
			First	Second	Third	Fourth	Fifth	Sixth
II	Thiamine deficient	25	0	40	68.0	100.0		
	Control	25	0	4	24 0	56.0	96.0	96.0
VIII	Thiamine deficient	24	0	0	8.3	29.1	50.0	87.5
	Control	24	0	0	4.1	8.3	33.3	79.1

the work of Badger and Masunaga who confirmed Lamb's results on incubation and dissemination. Supplements of thiamine chloride completely corrected the susceptibility. The influence of calcium, also noted by Lamb, proved to be indirect. The calcium depletion caused a deficiency in thiamine. Despite calcium depletion normal resistance to leprosy could be maintained by large amounts of thiamine. A representative measure of resistance was the time at which palpable lepromata appeared.

Many epidemics of typhus fever have been associated with periods of famine and a relationship between diet and typhus has been sought. Zinsser, Ruiz Castaneda and Seastone found that scurvy, in the guinea pig, increased the susceptibility to typhus. They inoculated their animals after symptoms of scurvy appeared. In such animals the rickettsiae were more widely distributed and more numerous than in adequately fed animals. Vitamin C deficient diets increased the susceptibility of rats also but not to the same extent.

More recent work (Pinkerton and Bessey) shows that riboflavin defi-

ciency is even more effective in reducing the resistance of rats to typhus fever. This study is of great interest because it represents the successful application of a known characteristic of a deficiency disease. Rickettsiae are intracellular organisms and as such are much affected by the health of their host cells. Cellular oxidation is reduced in riboflavin deficiency. Therefore conditions affecting the growth of rickettsiae might be presumed to occur during deficiency. This proved to be the case. The organisms were widely disseminated throughout the organs and were present in tremendous numbers.

More recently evidence has accumulated indicating that the vitamins are a double-edged sword. Bloomfield and Lew observed that deficient animals were more resistant to a natural disease (ulcerative caecitis) than adequately fed ones. Rasmussen et al. demonstrated that thiamine deficiency protected mice against paralysis when they were intracerebrally infected with mouse adapted poliomyelitis virus. This subject has been discussed in more detail in Chapter XVII. It is suggested that the nutritional requirements of both the parasite and host must be considered in estimating the significance of vitamin supply. Conditions will doubtless prove different for various pathogens. The possibility of transient depletion of the host of a nutrient essential to its parasite serving a useful therapeutic purpose will doubtless be energetically studied.

CHAPTER XXVI

MEDICAL CARE OF NUTRITIONAL FAILURE

The diagnosis and treatment of nutritional failure requires much of the knowledge and art of the practitioner. It is important that he *suspect* inadequate nutrition when organic disease of a kind which frequently predisposes to deficiency states is present, when the economic status of his patient implies that deprivation is probably present, when alcoholism or dietary idiosyncrasies are recognized. Suspicion can then be fortified by inquiry into the amounts of certain protective foods which the patient uses, milk, butter, eggs, vegetables, fruits, enriched cereals and breads.

The physical examination should regularly include examinations designed to reveal dietary deficiencies. The tongue and mouth are probably the most important. The conjunctiva and skin should also be inspected with deficiency lesions in mind. One of the check forms used by Spies (Fig. 45) may be useful as a reminder of the changes to be sought.

If the history and physical examination imply the presence of vitamin deficiency a formula may be prescribed containing the estimated daily requirements of thiamine, riboflavin, niacin and ascorbic acid. If specific lesions of a particular deficiency are present the daily requirements of the particular vitamin should be doubled or trebled until response has been secured.

Most nutritionists have continued to use liver extract, given parenterally, or brewer's yeast, 4 oz. daily, as a supplement to synthetic vitamins. There is a considerable weight of clinical opinion to justify this type of treatment. Cereal embryo is equally satisfactory as a supplement and is frequently preferred to brewer's yeast.

The conditions which predispose to the deficiency must be met with tact and good judgment. If they be organic in nature their correction is necessary to a cure of the deficiency disease. Only the poorest cannot provide adequate diets if they desire to do so and know how. The tables of vitamin values in the Appendix should be helpful in suggesting foods which are acceptable, inexpensive and good sources of the vitamins especially needed. In many cases attendance at one of the nutrition courses now being given in most parts of the country is a practical solution. The rules for good cooking offered by the Bureau of Home Economics are useful:

Don't stir air into foods while cooking.
Don't put them through a sieve while still hot.
Don't use soda in cooking green vegetables.

In boiling foods, raise the temperature to the boiling point as rapidly as possible.

Use as little water as possible.

Don't use long cooking processes such as stewing when shorter methods are feasible.

Don't throw away the water in which vegetables have been cooked. Use it in making gravies, sauces and soups.

Prepare chopped fruit and vegetable salads just before serving.

Start cooking frozen foods while they are still frozen.

Serve raw frozen foods immediately after thawing.

Intensive therapy is sometimes indicated, as for example in pellagra (see Chapter XIX). Intensive therapy is sometimes used to exclude vitamin deficiency as a causative factor in an obscure condition. Thus it is said that 75 mgm. of thiamine, 600 mgm. niacin and 100 mgm. of pyridoxin given in divided doses per day for a week regularly give definite results in neurological conditions which are at all responsive to vitamin therapy. Ascorbic acid is conveniently given in 1 gm. amounts daily or every second day. If parenteral administration is desirable this method of treatment is more convenient and acceptable. The use of a single large dose of vitamin D, referred to in Chapter XXII, is often justified when the physician has misgivings about the dependability of the parent or guardian. Vitamin A *should be prescribed in large amounts (50,000 I.U.) for long periods* since response is usually slow.

PART III

TECHNICAL METHODS, VITAMIN ASSAY AND VITAMIN VALUE OF FOODS

Chapter XXVII

VITAMIN ASSAY METHODS

Before vitamins were isolated and chemically identified our only means of estimating potency was the so-called "biological assay" using test organisms such as the rat, mouse, guinea pig, chick, dog, etc. This method is still employed. The U. S. Pharmacopeia still requires a rat test to establish A and D potency of fish liver oils and viosterol and also for thiamine chloride potency.

With the availability of purified vitamins, it became possible to develop tests based on color reactions and spectrophotometric data and to expand the biological methods to use of bacteria and yeasts, the so-called "microbiological" tests.

GROWTH TESTS

Vitamin Potency was originally expressed in arbitrary "units," the unit being, in general, an amount of vitamin source required to produce either a specified growth response or curative effect in a test animal such as the rat, guinea pig, chick, dog, etc.

With the availability of pure vitamins it became possible to convert these "units" into actual weight of vitamin either by comparison with effect of pure vitamins themselves or, with so-called reference standards whose weight content of the vitamin is known, e.g. U. S. Pharmacopeia Reference Cod Liver Oil containing 850 U S.P. units of A and 85 units of D; Reference Ascorbic Acid ampul containing 100 mgm. Ascorbic Acid; Reference Standard Thiamine hydrochloride ampul containing 50 mgm. thiamine hydrochloride.

All growth tests depend upon first depleting the test animal of all stored vitamin of the kind to be assayed but at the same time supplying in the basal diet all other factors necessary for normal growth so that growth *limitation* is due solely to deficiency of the particular vitamin and then feeding an amount necessary to restore growth and comparing this effect with that of a known amount of the vitamin.

The *curative* biological test differs from the growth test only in finding an amount necessary to cure a definite pathological condition induced by depletion and comparison of this dose with a known amount of vitamin.

Details of biological tests that have been developed for particular vitamins will be found in the references given in the following pages.

MICRO-BIOLOGICAL TESTS

Animal biological assays are, at best, time consuming. Proof that micro-organisms like higher organisms required particular vitamins for growth stimulated the use of such organisms for vitamin assays. Today micro-biological tests are extensively used for all the members of the B complex group of vitamins. Effect of the vitamin is measured either by actual increase in cells or in output of cells in fermentation or acid formation.

Details of procedure for such tests are given quite fully in the University of Texas Bulletin #4137, to which the reader is particularly referred. Collateral references will be found under each vitamin in the following pages.

SPECTROPHOTOMETRIC TESTS

With the availability of pure vitamins it also became possible to make physical and chemical characteristics bases for estimation of vitamin presence and quantity in natural sources. If a vitamin shows selective absorption of certain light rays with characteristic maxima it becomes a relatively simple matter to detect its presence and by determining the degree of absorption, obtain an estimate of the quantity present.

The spectrographic method was first worked out for vitamin A following Morton and Heilbron's report on the absorption spectrum of the vitamin in 1928. Dr. Katharine Coward in her "Biological Standardization of the Vitamins," gives the following clear explanation of the principles involved:

"Conversion of values found spectroscopically into International units of vitamin A activity"

"The intensity of absorption of an oil at 328 mμ is expressed in the form $E^{1\%}_{1cm.}$ = X. That is, the solution examined must be a 1 per cent solution of the oil (or the corresponding amount of unsaponifiable matter) in alcohol or cyclohexane, and the solution must be examined in a cell of 1 cm. thickness. E is the log. of the ratio between I_0, the intensity of the incident light and I, the intensity of the emergent light. Thus, suppose the value of $E^{1\%}_{1cm.}$ for a particular oil is given as I. This means that only 10 per cent of the incident light is transmitted by a depth of 1 cm. of a 1 per cent solution of that oil; for, given log. I_0/I = *I*, therefore (since log. *10* = *I*) the intensity of the incident light is ten times the intensity of the emergent light; [illegible] cm. of a 1 [illegible] Similarly if the value of $E^{1\%}_{1cm}$ were given as 1.3, [illegible] log. 20 = 1.3, the intensity of the incident light is twenty times the intensity of the emergent light; thus the emergent light [illegible] cm. of a 1 per cent solution of [illegible]

If a few more values are substituted for $E^{1\%}_{1cm.}$ and the corresponding percentages of light absorbed are determined and all the percentages so obtained are plotted against the corresponding values of $E^{1\%}_{1cm.}$ it is found that the relationship is curvi-

linear. This may appear disturbing until it is realised that the percentage of light absorbed is not proportional simply to the concentration of the absorbing substance. That the values for $^{1\%}_{1cm}E$ are proportional to the concentration of vitamin A in a solution (provided no interfering substances are present) may be deduced from the general formula

$$E = \log. I_o/I = ecd, \text{ where e is a constant}$$

for the substance under examination, c is the concentration and d is the thickness of the layer of solution examined. In the measurement of vitamin A, e is a constant, d is always 1 cm and therefore constant; therefore $E^{1\%}_{1cm.} = Kc$, that is $E^{1\%}_{1cm.} \times c$ or $E^{1\%}_{1cm}$ varies directly as c. Thus the measure, $E^{1\%}_{1cm.}$ may be taken as a measure of the vitamin A content of liver concentrates.

The Permanent Commission on Biological Standardisation adopted provisionally the factor 1600 for converting the E value of an oil into the biological value in terms of the International unit. The evidence on which the decision to do this was based is summarised in the Special Report, No 202, of the Medical Research Council. There has been some dispute as to the validity of this factor, and the matter is under consideration. An early report (Hume, 1937) of an extensive investigation of a halibut liver oil shows that there is, so far, no good evidence that the factor should be changed."

For details on this method and bases for selection of the conversion factor we suggest the article by McFarlan, Reddie and Merrill (Ind. and Eng. Chem. 29: 324, 1937). See also other references in the following pages.

COLORIMETRIC METHODS

It has also been found possible to produce colored compounds by reactions of vitamins with special reagents and by measurement of the intensity of these colors to estimate vitamin potency. Reactions of vitamin A and carotene with antimony trichloride, of thiamine with a diazotized aromatic amine, of niacin with cyanogen bromide and anilin, etc., are now bases for such tests, specific references to which will be found under specific vitamins in the following pages.

COLLATERAL ASSAY PROBLEMS

Development of ways and means to make these assays specific and quantitatively accurate has involved development of specific instruments of measurement and also special methods of handling to release the vitamins from their natural sources.

Space does not permit adequate discussion of these many problems and methods and for that reason we give in the following pages what we believe to be particularly pertinent references for the understanding and operation of methods applicable to particular vitamins; classified according to methods.

METHODS FOR INDIVIDUAL VITAMINS

Vitamin A

U.S.P. Biological Assay: U.S.P. Pharmacopeia XI, p. 478–482. Also First Supplement p. 91–95; Second Supplement p. 132–136. This is a modification of the original rat growth test of Sherman and Munsell; J. Am. Chem. Soc. 47: 1639, 1925.

Antimony Chloride Colorimetric Test: This test is a development of the original Carr-Price reaction between vitamin A and antimony chloride; the blue color developing being by its intensity a measure of concentration of the vitamin in a given source.

Carr F. H. and Price E. A., Biochem. J. 20: 497, 1926, Spectro-photometric Test: This test is based on the absorption by vitamin A of a specific wave length of light and determination of the extinction coefficient with suitable instruments. An excellent review of the spectrometric versus the colorimetric method is found in the following: Oser, B. L., Melnick, D., Pader, M.: Analytical Edition Industrial and Engineering Chemistry, 15: 717, 724, 1943.

One of the problems met in using the spectro- or colorimetric methods is that of extraction of the vitamin from the source and the solvents to use in preparing the extract for assay. The following references cover pertinent observations on these points:

(a) Solvents for Ultraviolet Absorption, Spectro-Method:
Zscheile, F. P., Nash, H. A., Henry, R. L., Green, L. F.: Analyt. Ed. Industrial and Engineering Chem., 16:83, 1944.
Zscheile, F. P., Beadle, B. W.: ibid 14:633, 1942.
Zscheile, F. P., Henry, R. L.: ibid 14:422, 1942.
Morgareidge, K : ibid 14:700, 1942.
Coy, N. H , Sassaman, H. L., Black, A. · ibid 15:441, 1943.
Morton, R. A · Chemistry and Industry 59:301, 1940.

(b) Extraction and Preparation of Carotene for Tests:
Guilbert, H. S.: Analyt Ed. Ind & Eng. Chem. 6:452, 1934.
Peterson, W. J., Hughes, J. S., Freeman, H. F.: ibid 9:71, 1937.
Moore, L. A.: ibid 12:726, 1940.
Peterson, W. J.: ibid 13:12, 1941.
Fraps, G. S., and Kunmerer, A R.: ibid 13:806, 1941.
Moore, L A , and Ely, R.: ibid 13:600, 1941.
Mackinney, G., Aronoff, S , Bornstein, B. T. · ibid 14:391, 1942.
White, J. W , Brunson, A M., Zscheile, F. P ibid 14:798, 1942.
Bischoff, E., Williams, K. T.: ibid 15:266, 1943
Haagen-Smit, A. J., Jeffrys, C. E. P., Kirchner, J G : ibid 15:179, 1943.
Wall, M. E., Kelly, E. G.: ibid 15:18, 1943.

N.B. The bibliography references in these papers will further extend contact with problems of extraction and assay in this field of methodology.

Vitamin B_1 (Thiamine)

One of the earliest methods of expressing vitamin B_1 potency was in Sherman-Chase units (Chase, E. F. and Sherman, H. C.: J. Am. Chem. Soc., 53: 3506, 1931). It was, like the vitamin A bioassay method, based on growth of rat response. Smith (Smith, M. I.: U. S. Public Health Report 45: 116, 1930) had also developed a rat curative test and the U.S.P. Pharmacopeia test is a development of the Smith method.

See U. S. Pharmacopeia XI, Second Supplement p. 129–132; 138.

In 1936 Jansen (Jansen, B. C. T.: Rec. trav. chim. 55: 1046, 1936) developed a test for vitamin B_1 which involved first conversion of the vitamin into thiochrome and then estimation of quantity by measurement of the fluorescence of the thiochrome. This test has been modified by various workers and the procedure of general acceptance today is that of Hennessy and Cerecedo (Hennessy, D. J., and Cerecedo, L. R.: J. Am. Chem. Soc. 61: 179, 1939).

Another type of colorimetric test was developed in 1939 using a reagent first suggested by Prebluda and McCollum (Prebluda, H. J., and McCollum, E. V.: Sci. 84: 488, 1936 and J. Biol. Chem. 127: 495, 1939) and depending on the formation of a pigment by coupling the vitamin with a diazotized aromatic amine. This test was further developed by Melnick and Field (Melnick, D., and Field, H.: J. Biol. Chem. 127: 505, 515, 531, 1939 and 130: 97, 1939).

In 1937 Schultz, Atkin and Frey (Schultz, A. S., Atkin, L., and Frey, C. N.: J. Am. Chem. Soc. 59: 948, 1937) reported a microbiological test using yeast as the organism.

Schopfer used *Phycomyces Blakesleeanus* (Schopfer, W. H.: Bull. Soc. chim. biol. Paris, 17: 1097, 1935) and Lwoff has employed the *Glaucoma piriformis* (Lwoff, A. M.: Compt. rend. soc. biol. Paris, 126: 644, 1937).

There have been other biological tests used including response of pigeons or chicks made polyneuritic on polished rice and also a heart effect, bradycardia (Baker, A. Z. and Wright, M. D.: Biochem. J. 32: 2156, 1938).

Articles related to these tests:

(a) For discussion of Relative Merits:
Henessy, D. J : Analyt. ed. Ind & Eng. Chem. 13: 216, 1941.
Moyer, J. C , Tressler, D. K.: ibid 14: 788, 1942.
Brown, R. A., Hartzler, E., Peacock, G., Emmett, A. D.: ibid 15: 494, 1943.

(b) Modifications of the Thiochrome Test:
Conner, R. T., and Straub, G. J.: Analyt. ed. Ind. & Eng. Chem. 13: 380, 1941.
Pader, M.: ibid 15. 25, 1943
Clausen, D. F., and Brown, R. E.: ibid 15: 100, 1943.
Hinman, W. F., Halliday, E. G., Brookes, M. H.: ibid 16: 116, 1944.

(c) *Modifications of the Yeast Microbiological Test:*
Schultz, A. S., Atkin, L., Frey, C. N.: J. Am. Chem. Soc. 63, 632, 1941.
Schultz, A. S., Atkin, L., Frey, C. N.: Analyt. ed. Ind. & Eng. Chem. 14: 279, 1942.
Bunzell, H. H.: ibid 14: 279, 1942.

(d) Bacteriological Microbiological tests:
Williams, R. J., McMahan, J. R., Eakin, R. E.: Univ. Texas Bull. #4137, Oct. 1, 1941.
Williams, R. J., McAllister, E. D., Roehm, R. R: J. B. C. 83: 315, 1929.
Williams, R. J., Roehm, R. R.: J. B. C. 87: 581, 1930.

(e) Modification of the Colorimetric Test:
Emmet, A. D., Peacock, G., Brown, R. A.: J. B. C. 135: 131, 1940.

Vitamin B_2 (G) (Riboflavin)

Up to recently the potency of riboflavin sources was expressed in Sherman-Bourquin units but today the Food and Drug Administration of the U. S. Department of Agriculture requires that the label state the actual weight of this vitamin. The Sherman-Bourquin unit was determined (Bourquin, A., and Sherman, H. C.: J. Am. Chem. Soc. 53: 3501, 1931) by rat growth test, a bioassay method. For equivalence of the Bourquin-Sherman unit see Table II.

Because of the fluorescence of riboflavin this property was made the basis of a test (Supplee, G. C. et al.: J. Dairy Sci. 19: 215, 1936) which is now in common use for more rapid determinations.

A microbiological test was devised by Snell and Strong (Snell, E. E., and Strong, F. M.: *Analyt. ed. Ind. & Eng. Chem. 11: 347, 1939*) which measures the influence on the riboflavin on the cell growth and acid production of lactobacillus casei grown on a synthetic medium free of flavin. See also University of Texas *Bulletin #4137, Oct. 1, 1941.*

For discussion of these methods the following articles are suggested.

(a) Comparison of the Three Methods:
Emmet, A. D., Bird, O. D., Brown, R. A., Peacock, G., and Vanderbelt, J. M.: Analyt. ed. Ind. & Eng. Chem. 13: 219, 1941.

(b) Modification of Rat Growth Methods:
Hoagland, R., and Snider, G. G.: J. Agric. Res. 41: 205, 1930.
Christensen F W, Lutske, E, Hopper, T. H.: J. Agric. Res. 53: 415, 1936.
Hoagland, R.: ibid 38: 431, 1939
Ittner, N. R., Hughes, E. H. Food Res. J. 6: 239, 1941.

(c) Fluorimetric Method Modifications:
Kemmerer, A. R.: J. Assoc. Off. Agric. Chemists 23: 346, 1940.
Peterson, W. J., Brady, D. E., Shaw, A. I.. Analyt. ed. Ind. & Eng. Chem. 15: 634, 1943

(d) [illegible]
[illegible] t. ed. Ind. & Eng. Chem. 14: 271,
[illegible] uppl. 31: 95, 1941.
Bauernfeind, J. C, Sotier, A. L., Boniff, C. S: Ind. & Eng. Chem. 14: 666, 1942.

Strong, F. M., and Carpenter, L. E.: ibid 14: 909, 1942.
Hodson, A. Z., and Norris, L. C.: J. B. C 131: 621, 1939.
Mickelson, O., Waisman, H. A , Elvehjem, C. A.: J. Nutrn. 18: 517, 1939
Kemmerer, A. R.: J. Assoc. Off. Agric. Chemists 24: 413, 1941.

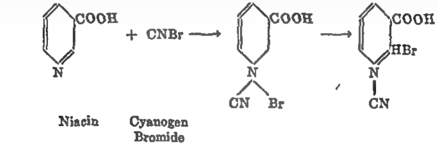
Niacin (Nicotinic Acid)

The disease known as "blacktongue" in dogs responds to the same curative agent as does human pellagra and the biological assay method for niacin is based on this fact.

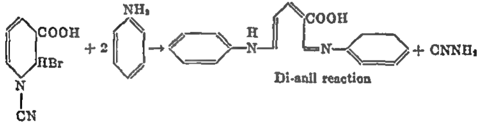
Elvehjem et al. (Elvehjem, C. A., Madden, R. J., Strong, F. M., and Wooley, D. W.: J. Am. Chem. Soc. 59: 1767, 1937 and J. Biol. Chem. 123: 137, 1939) used this method to establish the fact that niacin and niacin amide are pellagra preventive vitamins.

In 1904 Konig (Konig, W.: J. Prakt. Chem., 69: 105, 1904 n.t.) used cyanogen bromide to break the pyridine ring and liberate the nitrogen. The ring thus opened the reaction is followed by conjugation of the carbon chain with some aromatic amine to produce a stable colored compound.

(a)



(b)



This reaction can be also produced by other compounds than cyanogen bromide and other aromatic amines than aniline.

The use of cyanogen bromide and aniline now constitutes a colorimetric assay method which is discussed in detail by Waisman and Elvehjem (Waisman, H. A., and Elvehjem, C. A.: Analyt. ed. Ind. and Eng. Chem. 13: 221, 1941).

Snell and Wright (Snell, E. E., and Wright, L D.: J. Biol. Chem. 139: 675, 1941) developed a microbiological method using the *lactobacillus arabinosus* as the organism, and Stokes et al. (Stokes, J. L , Larsen, A.,

Woodward, C. R., Jr., and Foster, J. W.: J. Biol. Chem. 150: 17, 1943) use a mold, *neutrospora sitophila*. Still another organism, *Bacterium proteus* has also been used for assay of niacin (Lwoff, A., and Querido, A.: Compt. rendu Soc. biol. Paris, 129: 1039, 1938).

For data bearing on these methods the following articles are suggested:

(a) Modifications of the colorimetric method:
- Melnick, D., Robinson, W. D., Field, H., Jr.: J. Biol. Chem. 136: 131, 1940.
- Melnick, D., Field, H., Jr.: ibid 134: 1, 1941.
- Melnick, D.: Cereal Chem. 19: 553, 1941.
- Melnick, D., and Oser, B. L.: Analyt. ed. Ind. & Eng. Chem. 15: 355, 1943.
- Melnick, D., Oser, B. L., Siegel, L.: ibid 13: 879, 1941.
- Lamb, F. W.: ibid 15: 352, 1943.
- Greene, R. D., Black, A., Howland, F. O.: ibid 15: 77, 1943.
- Jones, W. S.: J. Am. Pharm. Assn. Sci. ed. 30: 372, 1941.
- Waisman, H. A., Elvehjem, C. A.: Anal Ed. Ind. and Eng. Chem., 13: 221, 1941.
- Dann, W. J., Handler, P.: J. B. C., 140: 201, 1941.

(b) Modifications of the microbiological method:
- Snell, E. E., Wright, L. D.: Univ. Texas Bull #4137, Oct. 1, 1941.
- Andrews, J. S., Boyd, H. M., Gertner, W. A.: Analyt. ed. Ind. and Eng. Chem. 14: 663, 1942.
- Cheldelin, V. H., Williams, R. R.,: ibid 14· 671, 1942.
- Isbell, H., Wooley, D. W., Butler, R. E., Sebrell, W. H.: J. Biol. Chem. 139: 499, 1941.

Vitamin B_6 (Pyridoxine)

György in 1934 (György, P.: Nature 133: 498, 1934) using a rat test defined a unit of pyridoxine as the amount necessary to cure acrodynia of moderate severity in three weeks. This was the first attempt at quantitative estimation of B_6 potency. Wilson and Roy (Wilson, H. E. C., and Roy, G. K.: Indian J. Med. Res. 25: 879, 1938) and Schneider et al. (Schneider, H. A., Asham, J. K., Platz, B. R., and Steenbock, H.: J. Nut. 18: 99, 1939) proposed slightly different units but all were based on a biological assay.

Colorimetric tests have also been proposed for this vitamin. (Swaminathian, M.: Nature 145: 780, 1940) and his test and others are discussed by Waisman and Elvehjem (Waisman, H. A., and Elvehjem, C. A.: Analyt. ed. Ind. & Eng. Chem. 13: 224, 1941).

Microbiological tests using yeast as the organism have also been developed, e.g. see:

- Schultz, A. S., Atkin, L., Frey, C. N : J. Am Chem. Soc. 61: 1931, 1939.
- Snell, E. E., Guirard, B. M., Williams, R. J.: J. Biol. Chem. 143: 519, 1942.
- Eakin, R. E., and Williams, R. J.: J. Am. Chem. Soc. 61: 1932, 1939.
- Williams, R. J., Eakin, R. E., McMahan, J. R.: Univ. Texas Publication 4137, 24: 1941.

Atkin, L., Schultz, A. S., Williams, W. L., Frey, C. N.: Analyt. ed. Ind & Eng. Chem. 15: 141, 1943.

See also Möller's (Moller, E. F.: Ztschr. f. physiol. chem. 260: 246, 1939) discussion of use of lactic acid bacteria and also Landy and Dicken (Landy, M., and Dicken, D. M.: J. Lab. and Clin. Med.; 27: 1086, 1942).

Pantothen (Pantothenic Acid)

Pantothen or Pantothenic Acid was discovered in a search for a bios factor. To date, microbiological methods of assay using yeast or *Lactobacillus casei* are the principal methods of assay.

(a) Yeast methods:

Atkin, L., Williams, W. L., Schultz, A. S., Frey, C. N.: Analyt. ed. Ind. & Eng. Chem. 16 67, 1944.

Mitchell, H. K., Weinstock, H. H., Snell, E. E., Staberry, S. R., and Williams, R. J.. J. Am. Chem. Soc 62: 1776, 1779, 1785, 1791, 1940.

Williams, R. J., Lyman, C. M., Goodyear, G. H., Turesdail, J. H., Holiday, D.: J. Am Chem. Soc. 55: 2912, 1933.

(b) Lactobillus casei methods:

Pennington, D. E., Snell., E. E., Mitchell, H. K., McMahan, F. R., Williams, R. J.: Univ Texas Bull. #4137, Oct. 1. 1941.

Pennington, D, Snell, E. E, Williams, R. J · J. Biol. Chem. 135: 213, 1940.

Cheldelin, V H, Williams, R J. Univ. Texas Publication 4237, 105, 1942.

Strong, F. M, Feeney, R E, Earle Analyt ed Ind. and Eng. Chem 13: 566, 1942.

Bauernfeind, J C., Sotier, A L., Boroff, C S.. ibid 14: 666, 1942.

Willerton, E., Cromwell, H W: ibid 14 603, 1942.

Frost, D. V. ibid 15 306, 1943.

Landy, M., and Dicken, D M.· J Lab. & Clin. Med. 27: 1086, 1942.

(c) Other organisms for microbiological tests·

Pelczar, M. J., and Porter, J R J. Biol. Chem. 139: 111, 1941.

(d) Chick method: Jukes, T H.. J B. C., 129: 225, 1939.

Inositol

The relation of Inositol to mouse alopecia was first reported by Wooley. Eastcott in 1928 (Eastcott, E V.: J Physiol. Chem. 32: 1094, 1928) first showed it to be a "bios" or yeast growth factor.

A yeast microbiological test has been developed for inositol and for details consult:

Williams, R. J, Stout, A. K., Mitchell, H. K., McMahan, J. R.: Univ. Texas Bull. #4137, Oct 1, 1941

Williams, R. J., Eakin, R. E., Snell, E. E.: J. Amer. Chem. Soc. 62: 1204, 1940.

Wooley, D. W.: J. Biol. Chem. 140: 453, 1941.

Paba (Para-amino-benzoic Acid)

The following chemical methods have been employed for the quantitative determination of Paba:

Bratton and Marshall's (Bratton, A. C., Marshall, E. K., Jr.: J. Biol. Chem. 128: 537, 1939) sulfonamide method which is based on coupling of diazotized Paba in acid solution with N-(1-naphthyl) ethylene diamine dihydrochloride to produce a red color. Kirch and Bergeim's (Kirch, E R, Bergeim, O.: J. Biol. Chem. 148: 445, 1943) production of a soluble pink to red compound by reacting diazotizo Paba with thiamine.

LaRosa's (LaRosa, W. V.: Proc. Soc. Exptl. Biol. and Med. 53: 98, 1943) use of p-dimethylamino benzaldehyde.

Wisansky, Grattan, Gawson and Ansbacher (unpublished data) have made use of the tyrosinase reaction to obtain a color compound usable for Paba assay.

Microbiological methods have also been developed. Rubbo et al. (Rubbo, S. D., Maxwell, M., Fairbridge, R. A., Gillespie, J. M.: Australian J. Exp. Biol. & Med. 19: 185, 1941) used *clostridium acetobutylecium.* Landy and Dicken (Landy, M., and Dicken, D. M.: J. Biol. Chem. 146: 109, 1942) used *acetobactu suboxydans.* Lewis (Lewis, J. C.: J. Biol. Chem. 146: 441, 1942) used *lactobacillus arabinosus* and Mitchell et al. (Mitchell, H. K., Isbell, E. R., and Thompson, R. C.: J. Biol. Chem. 147: 485, 1943) used *neurospora crassa nuitant:*

Vitamin H (Biotin)

For this vitamin the microbiological test today is in most common use. Snell et al. (Snell, E. E., Eakin, R. E., Williams, R. J.: Univ. Texas Bull. #4137, Oct. 1, 1941 and J. Am. Chem. Soc. 62: 175, 1940) describe a method using yeast as the test organism. Robbins and Bartley-Schmidt (Robbins, W. J., and Bartley-Schmidt, M.: Bull. Torrey Bot. Club 66: 139, 1939) use *Nematospora Gossypii* report a test specific for biotin if inositol is present in the basal medium. See also Kögl, F., and Fries, N.: Ztschr. of Physiol. Chem. 249: 93, 1937.

The lactobacillus is also used for biotin assay. (Shull, G. M., Hutchings, B. L., and Peterson, W H.: J. Biol. Chem. 142: 913, 1942).

See also: Schweigert, B. S., Nielsen, E., McIntire, J. M., and Elvehjem, A.: J. Nutrn, 26: 65, 1943. Peterson et al. used *clostridium butylecium* the organism (Peterson, W. H., McDaniel, L. E., and McCoy, J.: Biol. ıem. 1933: p. lxxv, 1940 and Snell, E. E., and Williams, R. J., J. Am. ıem. Soc. 61, 3594, 1939) and Lampen et al. (Lanpen, J. O., Bahler, P., and Peterson, W. H.: J. Nutrn. 23: 11, 1942) used this method to ;imate the biotin content of several foods.

For chick test see Hegsted, O., Oleson, J. J., Mills, R. R., Elvehjem, A., Hart, C. B.: J. Nut. 20: 599, 1940.

Choline

There are standard colorimetric tests for choline, e.g. *Rosenheim's* test (Hawk's Physiol. Chem.):

Prepare alcoholic extract and after evaporation apply Rosenheim's iodo-potassium iodide solution of Krant's reagent. With the former choline *periodide crystals* are formed and with the latter a bright brick red ppt.

Jacobi et al. (Jacobi, H. P., Barrman, C. A., and Meck, W. J.: J. Biol. Chem. 138: 571, 1941) describe a process used in choline assay of foods that involves extraction of choline with a mixture of equal parts of ethyl alcohol and ether and hydrolysis of the extract with barium hydroxide followed by colorimetric estimation of the choline as an acetone solution of the reinecke salt. See also:

Rhian, M., Evans, R J , and St. John, L. L: J. of Nutrn. 25: 1, 1943.
Engel, R. W.. ibid 25· 441, 1943.
Beattie, F. J. R.. Biochem J 30. 1554, 1936.
Engel, R. W : J. Biol. Chem 144. 701, 1942.
Fletcher, J. P , Best, C. H , and Solandt, O. M.. Biochem, J. 29: 2278, 1935.

Folic Acid

Mitchell and Snell (Mitchell, H. K., and Snell, E. E.: Univ. Texas 4137, 36: 1941) describe a microbiological test using *streptococcus lactis R.* as the test organism. See also the following references:

Mitchell, H. K , Snell, E. E , and Williams, R J.: J Am. Chem. Soc. 63: 2284, 1941.
Snell, E. E , and Mitchell, H K · Proc Natl. Acad Sci. 27: 1, 1941.
Snell and Peterson (Snell, E E , and Peterson, W. H.: J. Bact. 39: 273, 1940) used *L. casei*.

See also review of method by Mitchell and Williams.

Mitchell, H K , and Williams, R J. J Am. Chem. Soc., 66: 271, 1944.

Vitamin C (Ascorbic Acid)

At present chemical tests are in almost universal use for estimation of vitamin C potency. The first satisfactory biological test, using the guinea pig as the test animal was devised by Sherman et al. (Sherman, H. C., LaMer, V., and Campbell, H. L : J. Am. Chem. Soc. 44: 165, 1922) coupled with an autopsy score.

Hojer (Höjer, A.: Acta Pediat. 3: 8, 1924) studied tooth changes in scurvy and developed a method of assaying C potency by degree of tooth defects in 1924. For comparison of growth and tooth test see Eddy, W. H.: Am. J. Public Health 19: 1309, 1930.

The chemical tests most in vogue are modifications of the reaction be-

for the bio-assay procedure. The method of Brockman and Chen is based on the discovery that vitamin D forms with a saturated solution of antimony trichloride a pink color with a prominent band at 500 $\mu\,\mu$. For discussion of this method see:

Brockman and Chen: Z physiol. Chem. 241: 129, 1936.
Emmerie and van Eekelen: Acta brevia Neerland, Physiol, Pharmacol. Physiol. 6: Nos. 9–10, 133, 1936.
Milas, N. A., Heggie, R., Raynolds, J. A.: Analyt. ed. Ind. & Eng. Chem. 13: 227, 1941.

Ewing et al. (Ewing, D. T., Kingsley, G. V., Brown, R. A., Emmett, A. D.: Analyt. ed. Ind. & Eng. Chem. 15: 301, 1943) have listed and reviewed the physico-chemical methods. They report the following.

(1) Measurement of height of ultra violet absorption maximum at 265 mu mu.
Reerink, E., Van Wijk: Chem Weekblad. 29: 645, 1932.
Töpelmann, H., and Schupknecht, W.: Z. Vitaminforsch 4: 11, 1935.
Al Khin B Proc Soc. Sci Inst. Vitamin Res. U S.S.R. 3. No. 1, 28–29, 1941.
Marcussen, E.: Dansk. Tids Farm. 13: 141, 1939.

(2) Production of a lilac-red solution suitable for colorimetric determinations through heating a solution of the vitamin and pyrogallol in benzene, petroleum ether, or chloroform with a fresh solution of anhydrous aluminum chloride in absolute alcohol.
Halden, W., and Tzoni, H. Nature 137: 909, 1936; Naturwissenshaften 24: 296, 1936, Biochem. Z 287. 18, 1936.

(3) Production of a red color by action of HCl and aniline.
Shear, J.: Proc Soc Exp Biol. & Med 23. 546, 1925.
Criticised by Levine (Levine, J: Biochem. J 27: 2047, 1933) as non specific for D vitamins

(4) Color reaction between D in oils and phosphorus pentachloride.
Stoeltzner, W.: Münch. med Wochenschr. 75. 1584, 1928 but like (3) criticised Christensen (Christensen, E Münch. med Wochenschr. 75: 1883, 1928) as non-specific for D

(5) Green color produced by mixing an acetic acid solution of D with a 2 per cent solution of bromine in chloroform
Rutkovskii, L A.: Biokimiya 5 528, 1940

(6) Yellow color produced by boiling an alcohol solution of D with sodium nitrite and acetic acid and then made slightly alkaline.
Robinson, F. Chemistry and Industry 56. 191, 1937.

Ewing et al. also describe a physical-chemical method based on the Brockman-Chen reaction with a preliminary separation of interfering substance by chromatograph absorption.

Vitamin E (Alpha-tocopherol)

Evans and Burr (Evans, H. M., Burr, G. O.: Mon. Univ. Calif. 8: 1927) described the general procedure by bioassay with which to demonstrate

presence of vitamin E. Evans' rat unit (Evans, H. M., Murphy, E. A., Archibald, R. C., and Cornish, R. E.: J. Biol. Chem. 108: 515, 1935) was the smallest quantity of test substance which, with rats as test organisms, results in birth of young when given in a single dose prior to the fifth day of gestation and Evans and the Emersons (Evans, H. M., Emerson, O. H., and Emerson, G. A.: J. Biol. Chem. 113: 319, 1936) showed this unit equivalent to be equivalent to 2.5 mgm. of alpha-tocopherol.

Palmer (Palmer, L. S.: Analyt. ed. Ind. & Eng. Chem. 9: 427, 1937) describes the application of the bio-assay method in detail.

Emmerie and Engel (Emmerie, A., and Engel, C., Rec. trav. chim. 57: 1351, 1938 and 58: 895, 1939) have reported a colorimetric test based on reaction of tocopherol with $FeCl_3$ and alpha, alpha-dipyridyl reagent.

Karrer and Keller (Karrer, P., and Keller, H.: Helv. chim. acta., 21: 1161, 1938) claim accurate potentiometric titration of tocopherol with $AuCl_3$.

Vitamin K (K_1, K_2, Menadione)

Almquist and Stokstad (Almquist, H. J., and Stokstad, E. L. R.: Div. of Poultry Husbandry, Univ. Calif. 1937) and Dam and Schønheyder (Dam, H., and Schønheyder, F.: Biochem. J. 30: 890, 1936) describe a method of bio-assay using the chick as the test organism. (See also Almquist, H. J., and Stokstad, E. L. R.: J. of Nutrn. 14: 235, 1937).

Ansbacher also describes a chick method (Ansbacher, S.: J. of Nutrn. 17: 303, 1939) which with some modification has been also used by Thayer et al. (Thayer, S., McKee, R. W., Binkley, S. B., MacCorquadale, D. W., Doisy, E. A.: Proc. Soc. Exp. B & M 40: 478, 1939). See also Dann, F. P.: Am J Physiol. 123: 48, 1938.

Menotti (Menotti, A. R.: Analyt. et. Ind. & Eng. Chem. 14: 601, 1942) has proposed a colorimetric reaction depending upon the interaction of sodium pentacyano amine ferroate in alkaline solution with 4-amin-2-methyl-1-naphthol to produce a blue color.

Chapter XXVIII

LABORATORY TESTS USEFUL IN THE DIAGNOSIS AND STUDY OF THE VITAMIN DEFICIENCY DISEASES

It appears desirable to include a few methods entailing only the use of laboratory apparatus ordinarily at hand in the clinical laboratory and which are helpful in determining whether deficiency is present. More elaborate methods will be briefly mentioned and references cited. Many of the methods of assay listed in the preceding chapter are readily adaptable to clinical work. Attention is particularly invited to the Symposium on Vitamins presented before the joint meeting of the Divisions of Biological and Agricultural and Food Chemistry at the 100th Meeting of the American Chemical Society, Detroit, Michigan (Ind. and Eng. Chem., Anal. Ed., Vol. 13, No. 3, pages 209–31).

VITAMIN A

Colorimetric methods based on the Carr-Price reaction (Biochem. J. 20, 497 (1926)) are in common use

Assay can be made on the serum or plasma (there is little vitamin A in the blood corpuscles). An alcohol petroleum ether extract is used which is treated with a chloroform solution of antimony trichloride. The blue color produced is unstable hence a rapid reading with a photoelectric colorimeter is recommended

Reagents:

Chloroform, pure and dry

Antimony trichloride, 225 grams per liter of chloroform

Alcohol, ethyl, 95 per cent

Petroleum ether (Baker's C.P. benzine B.P. 20–40°C.)

(Care in protection from light and moisture is required in the preparation and use of reagents. Kroehn, C. J., and Sherman, W. C.: J. Biol. Chem. 132, 527 (1940).)

Method of Vitamin A and Carotenoids

The method described is that of May, C. D., Blackfan, K. D., McCreary, J. F., and Allen, F. H.: *Clinical Studies of Vitamin A in Infants and in Children*, Amer. Jour. Diseases of Children 59, No. 6, p. 1167, June 1940.

Preparation of the Extract of Blood Serum (or Plasma). Place 1 ml. of serum or plasma in a narrow mouth tube (approx. 12 x 100 mm., tight

cork stopper), add 3 ml. of 95 per cent ethyl alcohol, shake, add 2 ml. of purified petroleum ether, stopper tightly, and shake vigorously for ten minutes in order to obtain complete extraction and then centrifuge. A clear layer of petroleum ether extract will rise to top. This is used for the analysis.

Analysis of the Extract. The yellow color of the extract is due mainly to carotene and xanthophyll. Bile pigments are not extracted under the above conditions. Since the carotene and xanthophyll are usually present in about equal amounts, the determination of the total carotenoids gives a sufficiently accurate measurement of the carotene present for practical purposes. The determination of the carotenoids is necessary in the determination of vitamin A, in order that the measurement of the total blue color formed in the Carr-Price reaction may be corrected for that portion due to the carotenoids.

Center Settings. Filter 440 (maximum transmission at 440 mu) is placed in the microunit of the photoelectric colorimeter. The center setting of the instrument for 1 ml. of pure petroleum ether in a 1 ml. open type microabsorption cell is determined as described in the directions for operation of the Evelyn photoelectric colorimeter.[1]

A similar "center setting" is determined for the antimony trichloride reagent (22.5 per cent solution of antimony trichloride in anhydrous chloroform) with filter 620 (maximum transmission at 620 mu). It is important to redetermine the center settings immediately before each analysis. One ml. of the antimony trichloride reagent is pipetted into a 1 ml. open type cell. The pipette used should be calibrated to deliver exactly 1 ml. within 4 seconds, with its tip steadied against the side of the cell, 3 mm. from the top, the last drop being blown out with a gentle puff. This insures rapidity and accuracy, and avoids immersion of the tip of the pipet into the reacting mixture. All operations must be performed rapidly and accurately.

Carotenoids. With filter 440 in place and the instrument set at the appropriate "center setting" for pure petroleum ether, 1 ml. of the serum extract is measured into a 1 ml. open type cell in place in the cell holder. The cell is immediately placed and adjusted in the colorimeter and the galvanometer reading (G440) recorded. This reading is used in calculating total carotenoids present.

Vitamin A. The 1 ml. cell with contents is removed from the colorimeter. The petroleum ether extract is now evaporated to dryness by gently blowing dry air into the cell which is partially immersed in water at 40 to 45°C. The outside of the cell is wiped dry and polished with lens paper, and the cell replaced in the cell holder of the colorimeter. Filter 620 is put into the colorimeter, and the galvanometer is set at the "center setting"

[1] Evelyn, K. A.: J. Biol. Chem. 115: 63, 1936.

for the antimony trichloride reagent. 1 ml. of the antimony trichloride reagent is now pipetted into the cell, the latter immediately placed and adjusted in the colorimeter. The blue color reaches maximum intensity in a few seconds. The galvanometer reading (G620) is made at the point of maximum absorption of light, that is, maximum color formation.

Expression of Results. May units are calculated as follows:

$$L = 2 - \log G \text{ (galvanometer reading)}$$

$$\text{Units per 100 ml of serum} = L \times \frac{2}{\text{ml. serum analyzed}} \times 100$$

Since it has been determined that each carotenoid unit (440) produces 0.11 of the blue color of the (620) unit with antimony trichloride, the total carotenoid units are multiplied by 0.11. This product subtracted from the total blue color units gives the vitamin A units.

Example. 1 ml. of petroleum ether extract from 1 ml. of blood serum was analyzed.

G440 reading = 71
G620 reading = 81
L440 = 0 1487
L620 = 0.0915

May units (440) per 100 ml. = 0.1487 × 2 × 100 = 29.7 total carotenoids
May units (620) per 100 ml. = 0.0915 × 2 × 100 = 18.3
May units (620) per 100 ml from carotenoids = 29.7 × 0.11 = 3.3
May units (620) per 100 ml. from Vitamin A = 18.3 − 3.3 = 15.0.
Conversion of May units into micrograms and International Units

Carotenoids May Units × 3 = micrograms
1 microgram = 1.66 I.U.
Vitamin A May Units × 3 = micrograms
1 microgram = 2.85 I.U.

PYRUVIC ACID IN BLOOD PLASMA

The method involves the stabilization of pyruvic acid in the blood sample by addition of sodium iodiacetate followed by the formation of the 2,4-dinitrophenylhydrazone of pyruvic acid. This is extracted and made alkaline which develops the color subsequently measured in the photoelectric colorimeter. By comparison with values read from a standard curve, the pyruvic acid content of the unknown is determined.

Method

Reagents:

2N sodium hydroxide solution
25 per cent sodium monoiodo acetate solution, adjusted to pH 7.8 with NaOH

cork stopper), add 2 ml. of 95 per cent ethyl alcohol, shake, add 2 ml. of purified petroleum ether, stopper tightly, and shake vigorously for ten minutes in order to obtain complete extraction and then centrifuge. A clear layer of petroleum ether extract will rise to top. This is used for the analysis.

Analysis of the Extract. The yellow color of the extract is due mainly to carotene and xanthophyll. Bile pigments are not extracted under the above conditions. Since the carotene and xanthophyll are usually present in about equal amounts, the determination of the total carotenoids gives a sufficiently accurate measurement of the carotene present for practical purposes. The determination of the carotenoids is necessary in the determination of vitamin A, in order that the measurement of the total blue color formed in the Carr-Price reaction may be corrected for that portion due to the carotenoids.

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Carotenoids. With filter 440 in place and the instrument set at the appropriate "center setting" for pure petroleum ether, 1 ml. of the serum extract is measured into a 1 ml. open type cell in place in the cell holder. The cell is immediately placed and adjusted in the colorimeter and the galvanometer reading (G440) recorded. This reading is used in calculating total carotenoids present.

Vitamin A. The 1 ml. cell with contents is removed from the colorimeter. The petroleum ether extract is now evaporated to dryness by gently blowing dry air into the cell which is partially immersed in water at 40 to 45°C. The outside of the cell is wiped dry and polished with lens paper, and the cell replaced in the cell holder of the colorimeter. Filter 620 is put into the colorimeter, and the galvanometer is set at the "center setting"

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Iay units (620) per 100 ml. from carotenoids = 29.7 × 0.11 = 3.3
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Conversion of May units into micrograms and International Units

Carotenoids May Units × 3 = micrograms
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Analysis of the Extract. The yellow color of the extract is due mainly to carotene and xanthophyll. Bile pigments are not extracted under the above conditions. Since the carotene and xanthophyll are usually present in about equal amounts, the determination of the total carotenoids gives a sufficiently accurate measurement of the carotene present for practical purposes. The determination of the carotenoids is necessary in the determination of vitamin A, in order that the measurement of the total blue color formed in the Carr-Price reaction may be corrected for that portion due to the carotenoids.

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Vitamin A. The 1 ml. cell with contents is removed from the colorimeter. The petroleum ether extract is now evaporated to dryness by gently blowing dry air into the cell which is partially immersed in water at 40 to 45°C. The outside of the cell is wiped dry and polished with lens paper, and the cell replaced in the cell holder of the colorimeter. Filter 620 is put into the colorimeter, and the galvanometer is set at the "center setting"

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or the antimony trichloride reagent. 1 ml. of the antimony trichloride reagent is now pipetted into the cell, the latter immediately placed and adjusted in the colorimeter. The blue color reaches maximum intensity in a few seconds. The galvanometer reading (G620) is made at the point of maximum absorption of light, that is, maximum color formation.

Expression of Results. May units are calculated as follows:

$$L = 2 - \log G \text{ (galvanometer reading)}$$

$$\text{Units per 100 ml of serum} = L \times \frac{2}{\text{ml. serum analyzed}} \times 100$$

Since it has been determined that each carotenoid unit (440) produces 0.11 of the blue color of the (620) unit with antimony trichloride, the total carotenoid units are multiplied by 0.11. This product subtracted from the total blue color units gives the vitamin A units.

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cork stopper), add 2 ml. of 95 per cent ethyl alcohol, shake, add 2 ml. of purified petroleum ether, stopper tightly, and shake vigorously for ten minutes in order to obtain complete extraction and then centrifuge. A clear layer of petroleum ether extract will rise to top. This is used for the analysis.

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Carotenoids. With filter 440 in place and the instrument set at the appropriate "center setting" for pure petroleum ether, 1 ml. of the serum extract is measured into a 1 ml. open type cell in place in the cell holder. The cell is immediately placed and adjusted in the colorimeter and the galvanometer reading (G440) recorded. This reading is used in calculating total carotenoids present.

Vitamin A. The 1 ml. cell with contents is removed from the colorimeter. The petroleum ether extract is now evaporated to dryness by gently blowing dry air into the cell which is partially immersed in water at 40 to 45°C. The outside of the cell is wiped dry and polished with lens paper, and the cell replaced in the cell holder of the colorimeter. Filter 620 is put into the colorimeter, and the galvanometer is set at the "center setting"

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for the antimony trichloride reagent. 1 ml. of the antimony trichloride reagent is now pipetted into the cell, the latter immediately placed and adjusted in the colorimeter. The blue color reaches maximum intensity in a few seconds. The galvanometer reading (G620) is made at the point of maximum absorption of light, that is, maximum color formation.

Expression of Results. May units are calculated as follows:

$$L = [illegible] - \log G \text{ (galvanometer reading)}$$

$$\text{Units per 100 ml of serum} = L \times \frac{2}{\text{ml. serum analyzed}} \times 100$$

Since it has been determined that each carotenoid unit (440) produces 0.11 of the blue color of the (620) unit with antimony trichloride, the total carotenoid units are multiplied by 0.11. This product subtracted from the total blue color units gives the vitamin A units.

Example. 1 ml. of petroleum ether extract from 1 ml. of blood serum was analyzed.

G440 reading = 71
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Conversion of May units into micrograms and International Units

Carotenoids May Units × 3 = micrograms
1 microgram = 1.66 I.U.
Vitamin A May Units × 3 = micrograms
1 microgram = 2.85 I.U.

PYRUVIC ACID IN BLOOD PLASMA

The method involves the stabilization of pyruvic acid in the blood sample by addition of sodium iodiacetate followed by the formation of the 2,4-dinitrophenylhydrazone of pyruvic acid. This is extracted and made alkaline which develops the color subsequently measured in the photoelectric colorimeter. By comparison with values read from a standard curve, the pyruvic acid content of the unknown is determined.

Method

Reagents:

2N sodium hydroxide solution
25 per cent sodium monoiodo acetate solution, adjusted to pH 7.8 with NaOH

cork stopper), add 2 ml. of 95 per cent ethyl alcohol, shake, add 2 ml. of purified petroleum ether, stopper tightly, and shake vigorously for ten minutes in order to obtain complete extraction and then centrifuge. A clear layer of petroleum ether extract will rise to top. This is used for the analysis.

Analysis of the Extract. The yellow color of the extract is due mainly to carotene and xanthophyll. Bile pigments are not extracted under the above conditions. Since the carotene and xanthophyll are usually present in about equal amounts, the determination of the total carotenoids gives a sufficiently accurate measurement of the carotene present for practical purposes. The determination of the carotenoids is necessary in the determination of vitamin A, in order that the measurement of the total blue color formed in the Carr-Price reaction may be corrected for that portion due to the carotenoids.

Center Settings. Filter 440 (maximum transmission at 440 mu) is placed in the microunit of the photoelectric colorimeter. The center setting of the instrument for 1 ml. of pure petroleum ether in a 1 ml. open type microabsorption cell is determined as described in the directions for operation of the Evelyn photoelectric colorimeter.[1]

A similar "center setting" is determined for the antimony trichloride reagent (22.5 per cent solution of antimony trichloride in anhydrous chloroform) with filter 620 (maximum transmission at 620 mu). It is important to redetermine the center settings immediately before each analysis. One ml. of the antimony trichloride reagent is pipetted into a 1 ml. open type cell. The pipette used should be calibrated to deliver exactly 1 ml. within 4 seconds, with its tip steadied against the side of the cell, 8 mm. from the top, the last drop being blown out with a gentle puff. This insures rapidity and accuracy, and avoids immersion of the tip of the pipet into the reacting mixture. All operations must be performed rapidly and accurately.

Carotenoids. With filter 440 in place and the instrument set at the appropriate "center setting" for pure petroleum ether, 1 ml. of the serum extract is measured into a 1 ml. open type cell in place in the cell holder. The cell is immediately placed and adjusted in the colorimeter and the galvanometer reading (G440) recorded. This reading is used in calculating total carotenoids present.

Vitamin A. The 1 ml. cell with contents is removed from the colorimeter. The petroleum ether extract is now evaporated to dryness by gently blowing dry air into the cell which is partially immersed in water at 40 to 45°C. The outside of the cell is wiped dry and polished with lens paper, and the cell replaced in the cell holder of the colorimeter. Filter 620 is put into the colorimeter, and the galvanometer is set at the "center setting"

[1] Evelyn, K. A.: J. Biol. Chem. 115: 63, 1936.

for the antimony trichloride reagent. 1 ml. of the antimony trichloride reagent is now pipetted into the cell, the latter immediately placed and adjusted in the colorimeter. The blue color reaches maximum intensity in a few seconds. The galvanometer reading (G620) is made at the point of maximum absorption of light, that is, maximum color formation.

Expression of Results. May units are calculated as follows:

$$L = 2 - \log G \text{ (galvanometer reading)}$$

$$\text{Units per 100 ml of serum} = L \times \frac{2}{\text{ml. serum analyzed}} \times 100$$

Since it has been determined that each carotenoid unit (440) produces 0.11 of the blue color of the (620) unit with antimony trichloride, the total carotenoid units are multiplied by 0.11. This product subtracted from the total blue color units gives the vitamin A units.

Example. 1 ml. of petroleum ether extract from 1 ml. of blood serum was analyzed.

G440 reading = 71
G620 reading = 81
L440 = 0 1487
L620 = 0.0915

May units (440) per 100 ml = 0.1487 × 2 × 100 = 29.7 total carotenoids
May units (620) per 100 ml. = 0.0915 × 2 × 100 = 18.3
May units (620) per 100 ml. from carotenoids = 29.7 × 0.11 = 3.3
May units (620) per 100 ml from Vitamin A = 18.3 − 3.3 = 15.0.
Conversion of May units into micrograms and International Units

Carotenoids May Units × 3 = micrograms
1 microgram = 1.66 I.U.
Vitamin A May Units × 3 = micrograms
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PYRUVIC ACID IN BLOOD PLASMA

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[1] Evelyn, K. A.: J Biol. Chem. 115. 63, 1936.

Ascorbic Acid (Taylor, F. H. L., Chase, D., and Faulkner, J. M.: Biochem. J., 30, 1119, 1936).

Reagents:

1. Indicator solution of 2,6-dichlorophenol-indophenol, 0.2 mgm./ml.
2. Glacial acetic acid.
3. Standard solution of ascorbic acid, 1 mgm./ml.
4. Starch indicator, 0.5 per cent solution.
5. Iodine solution, 0.01 N.
6. Sodium thiosulfate solution, 0.1 N.
7. Bromate solution.
8. Metaphosphoric acid solution, 10 per cent.

Indicator Solution:

Dissolve 20 mgm. indophenol in 75 ml. boiling water and cool. Filter into a 100 ml. volumetric flask and add distilled water to make 100 ml. Dilute 1:10 for plasma determination, not for urine determination.

Replace this reagent within 2 weeks. Keep in a brown glass-stoppered bottle in the refrigerator.

Standardize against the standard solution of ascorbic acid as follows: From a 5 ml. microburet containing indicator solution titrate to a definite pink color lasting 30 seconds 0.2 ml. standard solution of ascorbic acid in a centrifuge tube to which a drop of glacial acetic acid has been added.

Standard Solution:

Dissolve 100 mgm. ascorbic acid in 100 ml. distilled water.

Keep in a brown glass-stoppered bottle in the refrigerator.

Standardize daily against the iodine solution as follows: From a 5 ml. microburet containing iodine solution titrate to a permanent blue color 1 ml. standard solution of ascorbic acid in a centrifuge tube to which a drop of 0.5 per cent starch solution has been added.

Iodine Solution:

Dissolve 20 to 25 g. potassium iodide in as little distilled water as possible. Add 12.7 g. iodine, and distilled water to make 1000 ml.

Dilute 1:10 to make 0.01 N iodine solution.

Standardize the 0.1 N iodine solution against 0.1 N sodium thiosulfate solution as follows: To 25 ml. iodine solution in a 250 ml. Erlenmeyer flask add sodium thiosulfate solution until nearly all the iodine has reacted as shown by the color. Add 5 ml. starch paste and titrate slowly until colorless.

Potassium oxalate (crystals)

10 per cent Monochloracetic acid aqueous solution

2,4-dinitrophenyl hydrazine solution. Prepare as a 0.1 per cent solution in 2N hydrochloric acid

Ethyl acetate

Nitrogen gas (cylinder)

Sodium carbonate solution

Pyruvic acid B.P. 55 to 60° at 10 mm., redistilled immediately before use.

Procedure: About 0.1 ml. of 25 per cent sodium iodoacetate (pH 7.8) is placed in a bottle containing 20 mg. of dry potassium oxalate, and 5 ml. of blood obtained by venipuncture is caught directly in the bottle. A 3 ml. portion of this sample is now transferred dropwise into an Erlenmeyer flask containing 12 ml. of a 10 per cent solution of trichloracetic acid, with continuous shaking. This mixture is allowed to stand for 30 minutes, filtered, and 3 ml. of the filtrate transferred to a test-tube to which 1 ml. of 0.1 per cent dinitrophenyl hydrazine in hydrochloric acid has been added. This is allowed to stand for ten minutes and is then extracted with 4 ml. of ethyl acetate, mixing the layers by blowing in a gentle current of nitrogen through a capillary pipette. After separation of the layers, the lower is withdrawn through the same pipette and transferred to another test tube, where the extraction process is repeated with a fresh 2 ml. portion of ethyl acetate. The lower layer is removed as before, and re-extracted with 2 ml. portions of ethyl acetate until the lower layer is completely colorless The ethyl acetate extracts are all combined, and extracted with 2 ml. of sodium carbonate solution using the mixing technique already described. This extraction is repeated twice with 2 cc. portions of fresh sodium carbonate each time; the combined sodium carbonate extracts are washed with 1 ml. of ethyl acetate, and the sodium carbonate solution transferred to a special tube designed for use in the Evelyn colorimeter. The solution is now treated with 4 ml of 2 N sodium hydroxide solution, shaken well, and allowed to stand for ten minutes. The intensity of color due to the phenylhydrazone of pyruvic acid is then determined in the photoelectric colorimeter, using Filter 520 (Bueding, E., and Wortis, H.: J. Biol. Chem., 133: 585, 1940).

VITAMIN C

Most of the methods in use for the determination of ascorbic acid are based on the reduction of 2,6-dichlor-phenol-indophenol and the consequent color change first reported by J. Tillmans, Z. Untersuch. Lebensin, 54, 33, 1927. The method of A. O. Bessey, J. Biol. Chem., 126, 771, 1938, utilizes the photoelectric colorimeter.

The following method gives good results: Determination of Plasma

Guerrant to collect milk for vitamin C determinations. The bottle was flushed with carbon dioxide and applied to the teat.

The fecal content of ascorbic acid may be determined if two determinations are made of a H_2S free aqueous extract, one before and the other after oxidation of the ascorbic acid by ascorbic acid oxidase (Chinn and Farmer).

Torrance devised a special technique for skin samples. The collagen was softened by heating with acetic and metaphosphoric acid in a sealed, evacuated tube. Thereafter the handling was as for blood plasma.[2]

The Estimation of Capillary Resistance

The resistance or fragility of the capillaries may be estimated by several methods.

Compression Test. The simplest procedure is to pinch a rectangular area of skin between the thumbs and forefingers of both hands and to note, after a period of one minute, whether petechiae are present and how numerous they are. This is a perfectly satisfactory method for establishing the presence of distinctly low capillary resistance.

Göthlin's Method. A popular procedure has been the Rumpel-Leede test in which the pressure within the capillaries is increased by constricting the arm veins. Göthlin modified the test and standardized the results. It requires a rubber arm band of the kind used with sphygmomanometers which is placed about the arm and inflated to a pressure less than the diastolic pressure of the pulse. Göthlin uses three pressures, 35, 50 and [illegible] mm. Hg to make the test more quantitative. The pressure is maintained for fifteen minutes.

To interpret the test a circular area is imprinted on the skin over the antecubital fossa with a rubber stamp and the number of petechiae in this area are counted. Using a circle of 60 mm. diameter Göthlin found that healthy Scandinavians had fewer than five petechiae when a pressure of 50 mm. Hg was used. More than eight petechiae are considered subnormal.[3]

Suction Cup Method. Negative pressure outside the capillaries may be used instead of heightened pressure within. By this means the pressure range may be increased and more quantitative results are possible.

The test may be conducted in a number of ways. A satisfactory method is to use a small bicycle pump connected to a manometer and a small glass cup which is placed on the skin area to be tested and by means of which

[2] Muselin, R R , Silverblatt, E , King, C. G., and Woodward, G. E , Am J. Cancer, 27: 707, 1936. Knight, C. A , Dutcher, R. A., and Guerrant, N. B., Science, 89: 183, 1939. Chinn, H., and Farmer, C. J., Proc Soc. Exper. Biol. & Med., 41: 561, 1939. Torrance, C. C., Science, 87 332, 1938.

[3] Göthlin, G. F., Skand Arch f. Physiol., 61: 225, 1931.

Sodium Thiosulfate Solution:

Dissolve 24.8 g. sodium thiosulfate in freshly boiled water, and add distilled water to make 1000 ml.

Replace this reagent within 2 weeks. Keep sterile.

Standardize against bromate solution.

Metaphosphoric Acid Solution:

Grind 10 g. metaphosphoric acid in a mortar and dissolve in 100 ml. cold distilled water.

Replace this reagent within 2 weeks. Keep in the refrigerator.

Procedure:

1. Collect 7 ml. blood (fasting patient) in a tube containing oxalate.
2. Centrifuge immediately for 5 minutes.
3. Transfer 2 ml. plasma to centrifuge tube. Add 2 ml. distilled water and 6 ml. 10 per cent metaphosphoric acid solution. Stir rapidly with a glass rod for 30 seconds, and then allow to stand for 3 minutes.
4. Filter through Whatman No. 40 paper.
5. Transfer 5 ml. filtrate to a 15 ml. centrifuge tube, and titrate rapidly with indophenol solution dropped from a 5 ml. microburet to a definite pink color lasting 30 seconds.
6. Run a blank simultaneously, and deduct its titration value from that of the filtrate, to obtain the true titration value.

Calculation:

$$\frac{0.88 \times (\text{ml iodine} \quad 1 \text{ ml standard})}{5 \times (\text{ml dye} \quad 0.2 \text{ ml standard})} \times 100 \times \text{ml. dye used for titration}$$

$$= \text{mg. ascorbic acid per 100 ml. plasma}$$

1 ml. 0.01 N iodine solution represents 0.88 mgm. ascorbic acid. The values equivalent to standard are determined as described on page 1.

Example:

$$\frac{0.88 \times 1.58}{5 \times 2.06} \times 100 \times 0.06 = 0.81 \text{ mg. \% plasma ascorbic acid.}$$

Range:

0.02 to 10.0 mgm. ascorbic acid per 100 ml. plasma.

The ascorbic acid concentration of animal tissues can best be determined if the tissues are ground in a mortar surrounded by dry ice (Muselin, Silverblatt, King and Woodward).

A special container surrounded by ice was used by Knight, Dutcher and

is useful. If the clotting time of the diluted plasma is not greatly increased the prothrombin concentration is not seriously reduced. Where there is any doubt of the patients safety treatment should be relied on rather than the results of the determination of clotting time.

A reliable thromboplastin solution is necessary. Quick recommended macerating portions of a rabbits brain with acetone and repeating the process until a dry granular powder was obtained. This could be stored for a week in a refrigerator without loss of potency. The solution was prepared by dissolving 0.3 gram of the powdered brain tissue in 5 cc. physiologic saline, incubating for 10 minutes at 45°C. and centrifuging slowly for 3 minutes.

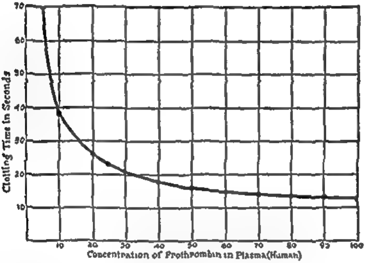

FIG. 46. Quick's figure for estimating prothrombin concentration from the clotting time. (Quick, A J., J. A M. A., 109. 66, 1937)

We have preserved thromboplastic preparations for several months by desiccation in a lyophile apparatus. Such preparations will probably be marketed by biological supply houses and should greatly simplify the Quick test. The limitations of the test have been referred to in the text.

Kato and Poncher have used this method in determining the clotting rates of infant's blood removed by means of a puncture wound of the heel and using one-tenth as much plasma, thromboplastin and calcium chloride solutions.[5]

[5] Quick, A. J., J. A. M. A , 110–1658, 1938 Smith, H. P., Ziffren, S. E , Owen, B. A. and Hoffman, G. R., J. A M A., 113: 380, 1939 Kato, K., and Poncher, H. G., J. A. M. A., 114: 749, 1940. Warner, E. D., Brinkhous, K. M , and Smith, H. P., Am. J. Physiol., 114: 667, 1936 Magath, T. B , Proc. Staff Meet. Mayo Clinic, 13: 67, 1938.

various negative pressures may be exerted on the capillaries. Since the capillary resistance varies in different regions of the body a small cup is preferable for a number of tests can be made in approximately the same region. We have used a cup with an inside diameter of 1 cm. and with a broad, flanged edge to prevent slipping of the skin.

The area of choice is the lateral aspect of the arm (not forearm) where the average resistance is approximately 30 ml. Hg, the center of the range of pressures possible with a hand pump. The cup is applied for one minute and the least negative pressure required to produce petechiae is determined by testing at several levels.

A practical method of reading the results is to determine the pressure necessary to produce several macroscopic petechiae easily seen with the unaided eye by ordinary light. By using a hand glass and by blanching the skin with a glass slide hemorrhages may frequently be found at lesser pressures but they are often difficult to identify positively. The simpler method of reading the test has therefore seemed to us to be more definite. The results should be verified by testing at higher and lower pressures.[4]

DETERMINATION OF PROTHROMBIN

Quick's method seems best suited to most clinical problems. Four and one-half cubic centimeters venous blood is mixed with 0.5 ml. of sodium oxylate solution (1.34 gram anhydrous sodium oxylate per 100 ml. water) and centrifuged. One-tenth ml. of plasma so obtained is mixed with an equal amount of thromboplastin solution and finally with 0.1 ml. of calcium chloride solution (1.11 grams anhydrous calcium chloride per 100 ml. distilled water). The time required for the formation of a clot after the calcium chloride solution is added is measured with a stop-watch. Normal plasma clots in 12 to 13 seconds. Various workers have found normal individuals with much longer clotting time. Very extensive testing of nearly a hundred persons revealed 25 to 30 seconds represents the upper limits of normality (Mason). Hemorrhage does not occur when the clotting time is 20 seconds or less. Hemorrhage may occur if the clotting time is between 45 and 100 seconds (Snell, Butt and Osterberg).

The explanation of this wide range of clotting time without a bleeding tendency is that the critical level of prothrombin concentration is only 20 to 30 per cent of normal. As pointed out in Chapter XXIV this represents a balance between rapid formation and destruction and is very unstable in patients with jaundice or hepatic diseases. Unfortunately the critical level cannot regularly be recognized by the clotting time test. Magath' suggestions of diluting the plasma with an equal amount of physiological saline and repeating tests in all cases in which the clotting time is 40 seconds or less

[4] Dalldorf, G., Am. J. Dis. Child., 46: 794, 1933.

COMPLETE BIBLIOGRAPHY

A simple determination can be made using whole blood. The sample (0.7 ml.) is discharged directly into a test tube containing 0.1 ml. of thromboplastin solution, the tube inverted once and tilted thereafter to reveal the time required for the formation of a clot. A normal blood should be timed as a control. The results are expressed as "clotting activity" which is expressed in per cent of normal. Values below 100 per cent indicate a bleeding tendency, hemorrhage will occur at values between 30 to 50 per cent (Smith, Ziffren, Owen and Hoffman).

The translation of clotting time into prothrombin values is facilitated by Quick's chart which is reproduced as figure 46.

The two-stage method of Warner, Brinkhouse and Smith is not adapted to clinical use. The authors' report should be consulted.

URINARY EXCRETION OF VITAMIN B_6[6]

The urine is made strongly alkaline (above pH 9.8 using thymol blue as indicator) with 30 per cent sodium hydroxide and allowed to stand overnight to destroy interfering reducing substances. The following morning 2 ml. samples are removed, about 45 ml. of water and 2 drops of half normal acetic acid are added. Adjust the pH to between 7.0 and 7.6 using phenol red on a spot plate. The volume is then brought to 50 ml.

To 5 ml. of the adjusted and diluted urine add 5 ml. of veronal buffer and 20 ml. of the chloramide in butanol solution. Stopper and shake vigorously. Allow to stand for 5 minutes and shake again. Allow to stand for 10 minutes. Centrifuge (three minutes will separate the layers). Wash the butanol layer twice with 10 ml. portions of the veronal buffer separating the layers by centrifugalization as above.

To 15 ml. of the washed butanol layer add 5 ml. of absolute alcohol, mix and read in the colorimeter. Our determinations have been made in a Cenco photometer using #4 filter and adjusting the instrument against a blank prepared from the patient's urine collected before administering pyridoxine. Hourly specimens are collected, the first one hour after the fasting patient has taken 250 ml. of water. This is discarded and a second sample collected one hour later during which time the patient drinks another 250 ml. of water. Three hourly specimens are collected after administering the vitamin. The excretion peak is reached within the first hour and falls rapidly after the second hour. If large amounts of vitamin are administered (5 mgm. per kg.) the excretion within three hours may exceed 25 per cent of the intake.

[6] Scudi, J. V., Unna, K., and Antopol, W., J. Biol. Chem., 135: 371, 1940

COMPLETE BIBLIOGRAPHY

CHAPTER I

HOPKINS, SIR GOWLAND: The Analyst 31: 385, 1906.
SCHOPFER, W. H.: Plants and Vitamins; Chronica Botanica Co., 1943, p. 41.

CHAPTER II

ALLISON, F. E., HOOVER, S. R., AND BURK, D.: Science 78: 217, 1933.
ANDERSAG, H., AND WESTPHAL, K.· Ber. d. deutsch chem. Gesellsch 70: 2035, 1937.
ANDERSON, R. J., AND NEWMAN, M. S.· J. Biol. Chem. 101: 773, 1933; ibid 103: 197, 1933
ANSBACHER, S.: Science 93: 164, 1941.
ASKEW, A. J.: Proc. Roy. Soc. London 109· 488, 1932.
BILLS, C. E.: J. Amer Med. Assn. 110· 2150, 1938.
BIRCH, T. W., GYÖRGY, P., AND HARRIS, L J : Biochem J. 29: 2830, 1935.
BLEYER, B., AND KALLMAN, O.· Biochem. Ztschr 155: 54, 1925.
BLOOR, W. R.: J. Am. Med. Assn. 119· 1018, 1942.
BLYTH, A. N.: J. Chem Soc 35· 530, 1879.
BOAS, M. A.: Biochem J 21: 712, 1927.
BOOHER, L. E.· J. Biol. Chem. 102 1105, 1938; ibid 119. 223, 1937.
BOOHER, L E : J. Biol. Chem 119. 223, 1937
BOURQUIN, A., AND SHERMAN, H C · J Am Chem Soc. 52: 3501, 1931.
CHICK, H., AND COPPING, A M Biochem J 24: 1764, 1930.
CLARKE, H. T., AND GURIN, S · J Am Chem. Soc 57. 1876, 1935.
DAM, H.· Biochem. Z 215 475, 1929 and 220· 158, 1930; also Biochem. J. 29: 1273, 1935.
EASTCOTT, E V : J. Physical Chem 32 1094, 1928.
ELVEHJEM, C. A , MADDEN, R J., STRONG, F M , WOOLEY, D. W.: J. Biol. Chem. 123: 137, 1939 See also, J Am Chem. Soc. [redacted]· 1767, 1937.
EMMETT, A. D., AND LUROS, G O . J. Biol Chem. 43 265, 1920
VON EULER, H., ALBERS, H , SCHLENK, F Z physiol. Chem. 237: I, 1935
EVANS, H. M., AND BISHOP, K [redacted] Am J. Physiol 63· 396, 1922.
EVANS, H. M., EMERSON, O H , AND G A · J Biol. Chem 113· 319, 1936.
FERNHOLZ, E . J. Am. Chem Soc 59. 1154, 1937 and ibid 60: 700, 1938.
FISCHER, G.· Ann. 127: 142, 1863
FOUTS, P. J., HELMER, O. M., LEPKOVSKY, J , AND JUKES, T. H.: Proc. Soc. Exp. B & M 37· 405, 1937
FRANCE, R., BATES, R. D , BARKER, W H., MATTHEWS, E.: Bull. Johns Hopkins Hosp 63: 46, 1938
FULMER, E J., DUECKER, W W , AND NELSON, V. E.: J. Am. Chem. Soc. 46: 723, 1923.
FUNK, C.: J. Physiol. 43: 395, 1931.
FUNK, C.: J. Physiol 46. 173, 1913.
FUNK, C., AND I. C.. J. Biol. Chem. 119: xxxv, 1937.
FUNK, C.: J. Am. Med Assn., Dec. 18, 1937.
GYÖRGY, P.: J. Am. Chem Soc. 60: 983, 1938.
GYÖRGY, P.: Ztschr. f. arztl Fortbild. 28: 377, 417, 1931.
GYÖRGY, P., AND ECKHART, E.: Nature 144: 512, 1939.

GYÖRGY, P., MELVILLE, D. B., BURK, D., AND DU VIGNEAUD, V.: Science 91: 243, 1940.

GYÖRGY, P., ROSE, C. S., HOFFMAN, K., MELVILLE, D. B., AND DU VIGNEAUD, V.: Science 92: 609, 1940.

HARRIS, L. J.: Chem. and Industry 56: 1134, 1937.

HARRIS, S. A., AND FOLKERS, K.: Science 89: 347, 1939 and J. Am. Chem. Soc. 61: 1237, 1242, 1939.

HARRIS, [illegible]. A., WOLF, D. E., MOZINGO, R., FOLKERS, K.: Science 97: 447, 1943.

HAWORTH, W. N., AND HIRST, E. L.: J. Soc. Chem. Ind. 52: 221, 1933.

HESS, A. F., ET AL.: J. Am. Med. Assn. 101: 181, 1933; J. Biol. Chem. 64: 181, 193, 1925; and ibid 62: 301, 1924.

HOGAN, A. G., AND RICHARDSON, L. R.: Bull. 241, Mo. Agric. Sta.—Science 83: 17, 1936.

HOSHIMO, T., AND OHTA, M.: Proc. Imp. Acad., Japan, 13: 101, 1937.

HUBER, C.: Ann. Chem. u. Pharm. 141: 271, 1867.

HUFF, J. W., AND PERLZWEIG, W. A.: Science 97: 539, 1943.

ICHIBA, A., AND MICHI, K.: Sci. papers. Inst. Phys. & Chem. Res., Tokyo, 34: 1014, 1938.

JANSEN, B. C. P., AND DONATH, W. F.: Mededeel. Dienst. Volkgezondheid, Nederland Indie, Part I, p. 186, 1926.

JUKES, T. H.: J. Am. Chem. Soc 61: 975, 1939.

KARRER, P., AND DEMOLE, V: Schweiz. med. Wochenschr. 68: 954, 1938.

KARRER, P., FRITSCHE, H., RINGIER, B. H., SALOMON, H.: Helv. chim. acta 21: 820, 1938.

KARRER, P., *HELFENSTEIN, A., WEHRLI, H., WETTSTEIN, A.: Helv. chim acta* 13: 1084, 1931.

KERESZTESY, J. C., AND STEVENS, J. R.: Proc. Soc. Exp. [illegible] & M. 38: 64, 1938.

KINNERSLEY, H. W., O'BRIEN, J. R. P., AND PETERS, R. A.: Biochem. J. 29: 2369, 1935.

KÖGL, F., AND TÖNNIS, B.: Ztschr. f. physiol. Chem. 242: 43, 1936.

KUHN, R., AND KALTSCHMITT, H.: Ber. d. deutsch Chem. Gesellsch. 68: 128, 1935.

KUHN, R., AND WENDT, G.: Ber. d. deutsch. Chem. Gesellsch 71: 780, 1938.

KUHN, R., AND WENDT, G.: Ber. d. deutsch Chem. Gesellsch. 71: 1118, 1938.

KUHN, R., GYÖRGY, P., WAGNER-JAUREGG, T.: Ber. d. deutsch Chem Gesellsch. *66: 317, 576, 1933*

KUHN, R., WAGNER-JAUREGG, T., VAN KLAVEREN AND VETTER: Z. physiol. Chem. 234: 196, 1935.

LEPKOVSKY, S Science 87: 169, 1938.

[illegible] J. Biol. Chem. 114: 109, 1936

[illegible] 115: 557, 1936.

[illegible]

[illegible] 941.

[illegible]

McCOLLUM, E V, AND KENNEDY, C.. J Biol. Chem. 24: 491, 1916.

MILLER, W. L, AND EASTCOTT, E V., AND MACHONACHIE, J E.: *J. Am Chem. Soc.* 55: 1502, 1933.

MITCHELL, H. H.: J. Biol Chem. 40: 399, 1919.

MITCHELL, H. K., AND WILLIAMS, R. J.: J. Am. Chem. Soc. 66: 271, 1944.

MITCHELL, H. K., SNELL, E E, AND WILLIAMS, R. J.: J. Am. Chem. Soc. 63: 2284, 1941.

Morgan, A. F.: Am J. Public Health 25: 328, 1935.
Morgan, A. F , and Simms, H. D.: J. Nutrn. 19: 233, 1940
Najjar, V. A., and Holt, L. E.: Science 93: 20, 1941.
Najjar, V. A., and Wood, R W.: Proc. Soc. Expl. B & M. 44: 386, 1940.
Najjar, V. A , Scott, A R McN , and Holt, L E : Science 97: 537, 1943.
Parsons, H. T., and Kelly, E.: Am. J. Physiol. 104: 150, 1933 and J. Biol. Chem. 100: 645, 1933
Perlzweig, W. A., Levy, E. D., Sarrett, H. P.: J. Biol. Chem. 136: 729, 1940.
Quackenbusch, F. W., Platz, B. R , Steenbock, H.: J. Nutrn. 17: 115, 1939.
Reichstein, T., Grussner, A., and Oppenhauer, R.: Helv. chim. acta Basel 16: 1019, 1933.
Rosenheim, O., and Webster, T.: Biochem. J. 21: 127, 1927.
Rubbo, S D., Maxwell, M., Fairbridge, R. A , Gillespie, J. M : Austral. J. Exptl. Biol. Med. 19: 185, 1941
Schneider, H A., Ascham, J K., Platz, B. R., Steenbock, H.: J. Nutrn. 18: 99, 1939.
Sebrell, W. H., Onstott, R. H., Fraser, G F , Daft, I. S.: J. Nutrn. 16: 355, 1938.
Shohl, A T., and Farber, S . J Nutrn 21. 147, 1941
Snell, E. E., and Peterson, W H.: J. Bact 39: 273, 1940.
Spies, T. D., Cooper, C , and Blankenhorn, M. A.: J. Amer. Med. Assn. 110: 623, 1938.
Spies, T. D., Grant, H. M , and Huff, N E.: So Med. J. 31: 901, 1938.
Steenbock, H.: Science 50: 352, 1919.
Stern, K. G., and Hofer, J W . Enzymologia 3. 82, 1937.
Stiller, E. T , Keresztesy, J. C , and Finkelstein, J.: J. Am. Chem. Soc. 62: 1779, 1940
Stiller, E. T., Harris, S. A., Finkelstein, J., Keresztesy, J. C., and Folkers, K.: J. Am Chem Soc. 62: 1785, 1940
Stokstad, E. L. R . J Biol Med 149, 573, 1943.
Sure, B.: Science 59: 19, 1924 and J Biol Chem 58. 693, 1924.
Sydenstricker, V P , Singal, S A , Briggs, A. P , DeVaughn, N. M., and Isbel, H.: Science 95: 176, 1942
Szent-Györgyi: Flexner Lectures, Series 6, 1939. Williams & Wilkins Co.
Tanret, M C.: Ann. chim phys , Series 5, 17 493, 1879
Tauber, H : J. Biol Chem. 123 499, 1938
Theorell, H.: Biochem Ztschr 272: 37, 155, 275, 1934, ibid 278: 263, 1935.
Todd, A. R , and Bergel, F : J Chem Soc. 1: 364, 1937.
du Vigneaud, V., Melville, D B , György, P., Rose, C. S.: Science 92: 62, 1940.
Wachenroder: Carotene, 1826.
Waddell, J : J. Biol Chem 105 711, 1934
Warburg, O , and Christian, W Biochem Ztschr 254: 438, 1932.
Warburg, O , and Christian, W. Biochem. Z. 274: 112, 1931; ibid 254: 438, 1932; ibid with Griese, 282. 157, 1935
Waugh, W. A., and King, C G : Science 75: 357, 1932; J Biol. Chem. 97: 325, 1932.
West, P. M., and Wilson, P. W : Science 89. 607, 1939.
Williams, R. J.: Biol Rev. 16 49, 1941 and Enzymologia 9: 387, 1941.
Williams, R. J , and Major, R. T.: Science 91: 246, 1940.
Williams, R. J , Lyman, C. M., Goodyear, G. H., and Truesdail, J. H.: J. Am. Chem. Soc. 55: 2912, 1933
Williams, R R., and Cline, J R. K.: J. Am. Chem. Soc. 58: 1504, 1936.

WILSON, H. E. C., AND ROY, G. K.: Indian J. Med. Res. 25: 879, 1938.
WINDAUS, A., AND HESS, A. F.: Nachr. ges. Wissensch. Goettinger, Math. Physik. Kl. 175: 1927.
WOODS, D. D.: Brit. J. Exptl. Path 21: 74, 1940.
WOODS, D. D., AND FILDES, P.: Chem. Industries 59: 133, 1940.
WOOLEY, D. W.: Science 92: 384, 1940.
WRIGHT, L. D., AND WELCH, A. D.: Science 97: 426, 1943.
WRIGHT, L. D., McMAHON, J. R., CHELDELIN, V. H., TAYLOR, A., SNELL, E. E., WILLIAMS, R. J.: Univ. Texas Bull. 4137: 38, 1941.

CHAPTER III

AMMON, R., AND DIRSCHEL, W.: Text Leipzig, G. Thieme, 1938.
BONNER, J., AND ADDICOTT, F.: Proc. Nat. Acad. Science 23: 453, 1937 and Bot. Gaz. 99: 144, 1938.
BONNER, J., AND BUCHMAN, E. R.: Proc. Nat. Acad. Sc. 24: 431, 1938.
BOTTOMLEY, W. P.: Proc. Roy. Soc., London, B, 88: 237, 1914 and ibid 89: 481, 1917.
VON EULER, H.: Erg. Vitamin & Harmonforschung 1: 159, 1938.
VON EULER, H., ALBERS, H., AND SCHLENK, F.: Z. physiol. Chem. 237: I, 1935.
FUNK, C.: J. Physiol. 43: 395, 1911.
FUNK, C.: The Vitamines, 1st Ed. 1913; 2nd. Ed. 1921, Williams & Wilkins Co.
LWOFF, A., AND M.: Proc. Roy. Soc., London, B, 122: 353, 1936.
MOCKERIDGE, F. A.: Proc. Roy. Soc., London, B, 89: 508, 1917.
NIELSEN, N., AND HARTELIUS, V.: C. r. trav. Lab. Carlsberg, serie physiologie, 22: No. 1, 1937.
SCHOPFER, W. H.: Translation by Noecker. Plants and Vitamins. Chronica Botany Co., 1943.
VILTER, R. W., VILTER, S. P., AND SPIES, T. D.: J. Amér. Med. Assn. 112: 420, 1939.
WARBURG, O., AND CHRISTIAN, W.: Biochem. Ztschr. 254: 438, 1932.
WILDIER, E.: La Cellule 18: 313, 1901.

CHAPTER IV

JOLLIFFE AND MOST, R. M.: Vitamins and Hormones, Vol. 1, pp. 59–107. Academic Press, 1943.

CHAPTER V

BOOHER, L. E., COLLISON, E. C., AND HEWSTON, E. M.: J. Nutrition 4: 323, 1939.
BESSEY, O. A., WOLBACH, S. B.: The Vitamins. Amer. Med. Assn., 1939.
GRAY, E., HICKMAN, K. C. D., AND BROWN, E. F.: J. Nutrition 19: 39, 1940.
HECHT, S., AND MANDELBAUM, J.: Am. J. Physiol. 112: 1910, 1939; also Hecht, S.: Physiol. Rev. 17: 273, 1937
McCOORD, A. B., AND LUCE-CLAUSEN, E. M.: J. Nutrition 7: 557, 1934
POPPER, H., AND GREENBERG, R.: Arch. Pathol. 32: 11, 1941.
SHERMAN, H. C.: Chemistry Food & Nutrition, 1941 ed., Macmillan, p. 421.

CHAPTER VI

AUHAGEN, E.: Ztschr. f. physiol. Chem. 204: 149, 1932; 209: 20, 1932.
BONNER, J., AND BUCHMAN, E. R.: Proc. Natl. Acad. Sci. U. S. A., 24: 431, 1938.
COWGILL, G. R.: The Human Requirement for Vitamin B. Yale Univ. Press, 1934.
FUNK, C., AND VON SCHONBORN, E.: J. Physiol. 48: 328, 1914.
GOODHART, R. S., AND SINCLAIR, H. M.: Biochem. J. 33: 1099, 1939.

JOLLIFFE, N.: New International Clinics. Vol. 10, Series 48, 1938. J. B. Lippincott Co.

LIPTON, M. A., AND ELVEHJEM, C. A.: Cold Spring Harbor Symp. Quant. Biol. 7: 184, 1939.

LOHMAN, K., AND SCHUSTER, P.: *Biochem. Ztschr.* 294: 188, 1937.

NEUBERG, C., AND KARCZAG, L.: Biochem. Ztschr. 36: 68, 76, 1911.

OCHOA, S.: Biochem. J. 33: 1262, 1939.

PETERS, R. A.: Chemistry at the Centenary (1931). The Vitamin B Complex.

SCHULTZ, F.: Ztschr. f. physiol. Chem 265: 113, 1940.

WEIL-MALHERBE, H.: Biochem. J 34: 980, 1940.

WILLIAMS, R. R., AND SPIES, T.: Vitamin B_1. Macmillan, 1938.

CHAPTER VII

AKROYD, W. R.: Biochem J. 24: 1479, 1930.

BALL, E. G: Symposium on Respiratory Enzymes. Univ. Wisconsin Press, 1942.

BANGA, I., AND SZENT-GYÖRGYI, A: Biochem. Ztschr. 246: 203, 1932.

BOURQUIN, A., AND SHERMAN, H C: J Am. Chem. Soc. 52: 3501, 1931.

GOLDBERGER, J., AND TANNER, W F.: Pub Health Reports 40: 58, 1925.

GURIN, S, EDDY, W. H., DENTON, J, AMMERMAN, M.: Exper. Med. 54: 421, 1931.

HOGAN, A. G.. J. Amer Med Assn. 110 1188, 1938

KOEHN, C. J., AND ELVEHJEM, C A: J Nutrition 11: 67, 1936.

KUHN, R., AND BOULANGER, P Ztschr f. physiol Chem 241: 233, 1936.

KUHN, R., GYÖRGY, P, AND WAGNER-JAUREGG, T.: Ber. d deutsch. Chem. Gesellsch. 66. 317, 576, 1933.

SNELL, E. E, AND STRONG, F M. Enzymologia 6: 186, 1939.

SURE, B., SMITH, M. E., KIK, M C, AND WALKER, D. J.: J. Biol. Chem 92: 8, 1931.

THEORELL, H.: Biochem. Ztschr 272. 37, 155, 275, 1934 and 278: 263, 1935.

WARBURG, O., AND CHRISTIAN, W: Biochem Ztschr 254: 438, 1932.

CHAPTER VIII

BUCHNER, E.: Ber. d. deutsch chem Gesellsch 30: 117, 1897.

HARDEN, A, AND YOUNG, W J. Proc Chem Soc 21: 189, 1905.

VON EULER, H., ALBERS, H, AND SCHLENCK, F Ztschr. f. physiol. Chem. 237: 1, 1935.

HUBER, C.: Ann. chem u Pharm 141: 271, 1867

PERLZWEIG, W. A, LEVY, E D, SARETT, H P J Biol. Chem. 136: 729, 1940.

VILTER, R. W., VILTER, S G, AND SPIES, T D.. J. Amer. Med. Assn. 112: 420, 1939.

ELVEHJEM, C. A.: Physiol Rev 20: 249, 1940

LANDY, M.: Proc. Soc. Exp. B & M 38 504, 1938.

CHAPTER IX

BIRCH, T. W.: J. Biol. Chem 124 725, 1938.

BIRCH, T. W., AND GYÖRGY, P. Biochem. J 30: 304, 1936.

BONNER, J., AND DEVIVIAN, P. S.: Am. J. Bot 26: 661, 1939

CHICK, H., AND COPPING, A. M.: Biochem J 24: 1764, 1930.

GYÖRGY, P.: J. Am Chem. Soc. 60: 983, 1938.

GYÖRGY, P.: Nature 133, 498, 1934 See also, György, P. and Eckhart, R. E.: Nature 144: 512, 1939.

HARRIS, S A., AND FOLKERS, K.: Science 89: 347, 1939 and J. Amer. Chem. Soc. 61: 1237, 1242, 1939

Ichiba, A., and Michi, K.: Sci. Papers Inst. Phys. Chem. Res. (Tokyo) 34: 1014, 1938.

Keresztesy, J. C., and Stevens, J. R.: Proc. Soc. Exp. B. & M. 38: 64, 1938.

Kuhn, R., and Wendt, G.: Ber. d. deutsch chem. Gesellsch. 71: 1118, 1938.

Lepkovsky, S.: J. Biol. Chem. 124: 125, 1938.

Moller, E. F.: Ztschr. f. physiol. Chem. 254: 285, 1938; ibid 260: 246, 1939. Also Angewandte Chemie [illegible]: 204, 1940.

Ohdake, 1931.

Robbins, W. J., and Bartley-Schmidt, M.: Am. J. Botany 26: 149, 1939.

CHAPTER X

Allison, F. E., Hoover, S. R., and Burk, D.: Science 78: 217, 1933.

Ansbacher, S.: Science 93: 164, 1941.

Axelrod, A. E., Gross, P., Bosse, M. D., and Swingle, K. F.: J. Biol. Chem 148: 721, 1943.

Best, C. H., and Ridout, J. H.: Ann. Rev. of Biochem. 1939; see also Science 94: 523, 1941.

Best, C. H., Channon, H. J., and Ridout, J. H.: J. Physiol. 81: 409, 1934.

Block, B., and Schaaf, F.: Biochem. Z. 162: 181, 1925, 1917.

Boas, M. A : Biochem. J. 21: 712, 1927.

Briggs, G. M., Jr., Luckey, T. D., Mills, R. C., Elvehjem, C. A., and Hart, E. B.: Proc. Soc. Exp. B. & M. 52: 7, 1943.

Briggs, G. M., Jr., Luckey, T. D., Elvehjem, C. A., and Hart, E. B.: J. Biol. Chem. 148: 163, 1943.

Daft, F. S., and Sebrell, W. H.: Pub. Health Report 58: 1542, 1943.

Day, P. L., Langston, W. C. and Shukers, C. F.: J. Nutrn. 9: 637, 1935.

Day, P. L., Langston, W. C., and Darby, W. J.: Proc. Soc. Exp. B. & M. 38: 860, 1938.

Day, P. L., Darby, W. J., and Langston, W. C.: J. Nutrn. 17: 13, 1939.

Day, P. L., Langston, W. C., Darby, W. J., Wahlin, J. G., and Mims, V.: J. Exper. Med. 72 463, 1940.

Dragstedt, L. R., Van Prohaska, J., and Harms, J. P.: Am J. Physiol. 117: 175, 1936.

Eakin, R. E., and E. Science 96: 187, 1942.

Eakin, R. E , Snell, E. E., and Williams, R. J.: J Biol. Chem. 140: 535, 1941 and ibid 136 801, 1940.

Eastcott, E. V.: J. Physical Chem 32: 1094, 1928.

Fulmer, E. J., Duecker, W. W., and Nelson, V. E.: J. Amer. Chem. Soc. 46: 723, 1923.

Gavin, G., and McHenry, E. W.: J. Biol Chem. 139: 485, 1941.

György, P.: J. Biol Chem. 131: 733, 1939; see also ibid 131: 745, 1939.

Gyorgy, P., and Poling, C. E.: Science 92: 202, 1940.

György, P , Melville, D. B., Burk, D. and du Vigneaud, V.: Science 91 · 243, 1940; see also Science 92: 62, 609, 1940.

György, P., Rose, C. S., Eakin, R. E., Snell, E E., Williams, R. J. Science 93: 477, 1941.

Griffith, W. H , and Wade, W. J.. J Biol. Chem 132 · 627, 1940, see also pp 169–183 in The Biological Action of the Vitamins, Univ. of Chicago Press.

Hogan, A. G. and O'Dell, B. L.: Science 97: 404, 1943.

Hogan, A G , and Parott · J. Biol. Chem. 132. 507, 1940.

Hogan, A. G., and Parrott, E. M : J. Biol. Chem. 128: Proc xlvi, 1939.

Hutchings, B. L., Bohonos, N., Hegstedt, Elvehjem, C. A., and Peterson, W. H.: J. Biol. Chem 140: 681, 1941.
Hutchings, B. L., Stokstad, E. L. R., Bohonos, N., Olesonn, J. J. and McElroy, L. R.: Report at 107th Meeting Am. Chem Soc., Apr. 1944.
Hutchings, B. L., Stokstad, E. L. R., Bohonos, N., Slobodkin, N. H.: Science 99: 371, 1944
Kerestztesy, J., Rickes, E. L., and Stokes, J. L.: Science 97: 465, 1943.
Kögl, F., and Tönnis, B.: Zeitschr. f. physiol. Chem. 242: 43, 1936.
Langston, W. C., Darby, W. J., Strukers, C. F., and Day, P. L.: J. Exper. Med. 68: 923, 1938.
Lepkovsky, S., Jukes, T. H., and Krause, M. E.: J. Biol. Chem. 115: 557, 1936.
Luckey, T. D., Briggs, G. M., Jr., and Elvehjem, C. A.: J. Biol. Chem. 152: 157, 1944.
Lunde, G., and Kringstad, H.: Ztsch f physiol. Chem. 257: 201, 1939.
Martin, G. J.: Proc Soc Exp B & M 51: 353, 1942.
Martin, G. J.: Fed Proc. 1: 58, 1942.
Martin, G. J.: Proc Soc Exptl B & M 51: 353, 1942.
Martin, G. J., Wisansky, W. A., and Ansbacher, S.: Proc. Soc. Exp. B & M. 47: 26, 1941.
Miller, W. L., Eastcott, E. V., and Sparling, E. M.: Trans. Roy. Soc. of Canada; III, 26: 165, 1932 See also J Chem. Education 7: 257, 1930 and J. Am. Chem. Soc. 55 1502, 1933.
Mitchell, H. K.: J Am. Chem Soc. 66. 274, 1944.
Mitchell, H. K., Snell, E. E., and Williams, R. J.: J. Am. Chem. Soc. 62: 2284, 1941. See also ibid 66: 267, 269, 271, 274, 1944
Morgan, A. F., Cook, B. B., and Davison, H. G.: J. Nutrition 15: 27, 1938.
Nielsen, R., and Elvehjem, C. A.: J Biol Chem. 145: 713, 1942.
O'Dell, B. L. and Hogan, A. G.: J. Biol Chem. 149. 323, 1943.
Parsons, H. T.: J. Biol Chem 90: 351, 1931. See also J Biol. Chem. 100: 645, 1943; 105: 1, 1934, 116 685, 1936, 123 xci, 1938; and J Nutrition 8: 57, 1934 and 19: Suppl. 19, 1940
Pavcek, P. L., and Baum, H. M.: Science 93: 502, 1941.
Pfiffner, J. G., Binkley, S. B., Bloom, E. S., Brown, R. H., Bird, O. D., Emmett, A. D., Hogan, A. G., Odell, B. L.: Science 97: 404, 1943.
Schaefer, A. E., McKibben, J. M., and Elvehjem, C. A.: Proc. Soc. Exptl. B & M. 47: 365, 1941.
Sieve, B. F. The Apothecary Vitamin Manual, April 1943 and So. Med. & Surg. 104: 135, 1942.
Snell, E. E., and Peterson, W. H.: J. Bacteriology 39: 273, 1940.
Spies, T. D., Stanbery, S. R., Williams, R. H., Jukes, T. H., and Babcock, S. H.: J. Amer Med Assn 115: 523, 1940. See also ibid 115: 292, 1940.
Stokes, J. L.: J Bact. 47. 27 (Proc) 1944
Stokstad, E. L. R.: J Biol Chem, 149: 573, 1943.
Stokstad, E. L. R. and Manning, P. D. V.: J. Biol. Chem. 125: 687, 1938.
Subarrow, Y. and Rane, L.: J. Amer. Chem. Soc. 61. 1616, 1939.
Totter, J. R., Shukers, C. F., Koeson, J., Mims, V., and Day, P. L.: Fed. Proc. Am Soc. Exper. Biol. 2: 72, 1943; J. Biol. Chem. 152: 147, 1944
Tschesche, R., and Wolf, H. J.: Stschr. f. physiol. Chem. 248: 34, 1937.
Unna, K., and Geeslin, J.: Proc. Soc. Expt. B & M. 45: 311, 1940.
duVigneaud, V.: The Biological Action of the Vitamins, Univ. Chicago Press. Biotin pp. 144–167.

DUVIGNEAUD, V., CHANDLER, J. P., MOYER, A. W., KEPPEL, D. M.: J. Biol. Chem. 131: 57, 1939.
WAISMAN, H. A., AND ELVEHJEM, C. A.: J. Nutrition 26·361, 1943.
WIEDER, S.: Present Status L. Casei Factor. Review reprint of Lederle Laboratories, 1944.
WILDIER, E.: La Cellule 18: 313, 1901.
WILLIAMS, R. J.: J. Biol. Chem. 38: 465, 1919.
WILLIAMS, R. J.: Science 29: 486, 1939.
WILLIAMS, R. J.: *Biol. Reviews 16: 49, 1941.*
WILLIAMS, R. J., AND MAJOR, R. T.: Science 91: 246, 1940.
WILLIAMS, R. J., LYMAN, C. M., GOODYEAR, G. H., TRUESDAIL, J. H.: J. Am. Chem. *Soc. 55: 2912, 1933.*
WILLIAMS, R. J., MOSHER, W. A., AND ROHRMAN, E.: Biochem. J. 30· 2035, 1936, 1940, strep lacti.
WILLIAMS, R. J., WEINSTOCK, H. H., JR., ROHRMAN, E., TRUESDAIL, J. H., MITCHELL, H. K., AND MYER, C. E.: J. Am. Chem. Soc. 61: 454, 1939..
WOOLEY, D. W.: Science 92: 384, 1940. Also J. Biol. Chem. 136: 113, 1940.
WOOLEY, D W., WAISMAN, H A., AND ELVEHJEM, C. A.: J. Am. Chem. Soc. 61: 977, 1939 and J. Biol. Chem. 129. 573, 1939.
WRIGHT, L. D., AND WELCH, A. D.: Science 97: 426, 1943.
WRIGHT, L. D , AND WELCH, A. D.: Science 98: 179, 1943.
WRIGHT, L. D., AND WELCH, A. D.. Am. J M. Sc. 206: 128, 1943.
WRIGHT, L. D., AND SKEGGS, H. R.: Proc Soc. Exper. B & M. 55: 92, 1944.
WRIGHT, L D., SKEGGS, H. R. AND WELCH, A. D.: Proc. Am. Soc. Exper. Biol. 3: 88, 1944.

CHAPTER XI

ARMENTANO, L., BENTSATH, A., BERES, T., RUSZNYAK, S , AND SZENTY-GYORGYI, A.: Deutsch med. Wochenschr. 62: 1325, 1936. See also, Nature 138. 798, 1936.
BEZSSONOFF, N.· Bull Soc. Chim Biol. 9· 568, 1917 and ibid 11: 294, 1929.
BORSOOK, H., DAVENPORT, H W , JEFFREYS, C. E. P., WARNER, R. C.: J Biol. Chem. 117: 237, 1937.
BUNDESEN, H. N., ARON, H C. S , GREENEBAUM, R S., FARMER, C. J., ABT, A. F.: *J. Amer. Med. Assn 117: 1692, 1941.*
CHICK, H. AND HUME, E.: Proc. Roy. Soc., London, 90· 44, 1917–19.
COHEN, B., AND MENDEL, L. B : J Biol Chem. 35: 425, 1918.
DAINOW, J.: Presse med 45: 1670, 1937.
ECKER, E. E., PILLEMER, L., GRIFFITHS, J. J., AND SCHWARTZ, W. P.: J. Amer. Med. Assn. 112: 1449, 1939.
EDDY, W. H., AND KOHMAN, E. F.: J Ind. & Eng. Chem. 16: 52, 53, 1924.
EVELYN, K. A., MALLOY, H T., AND ROSEN, C.: J. Biol. Chem. 126: 645, 1938.
FARMER, C. J , AND ABT, A F.: Proc. Soc. Exp. B & M 34: 146, 1936; See also J. Amer. Med. Assn. 111· 1555, 1938.
FUNK, C.: Die Vitamine. Wiesbaden, 1914.
HARRIS, L. J., AND RAY, S. N · Biochem. J. 27: 580, 1933 See also ibid 28: 996, 1934.
VON HAUSEN, S : Ann. Acad Sc Fennicae, Series A, 46, 134, 1935.
HAWORTH, W. N., AND HIRST, E L : J. Soc. Chem. Ind 52· 221, 481, 1933.
HÖJER, A · Studies in Scurvy, Acta pediat. 8 (Suppl. 3) 1924.
HOLMES, H N.: So Med & Surgery 104· Sept. 1943.

HOLST, A., AND FRÖHLICH, T.: Z. Hyg. Infektionskrankh. 72: 1, 1912; ibid 75: 334, 1913.
JACKSON, L., AND MOORE, J. S.: J. Infect. Dis. 19: 478, 1916.
KENNY, C. L.: Diss. Columbia Univ. 1926.
KING, C. G.: Ann. Rev. Biochemistry, 1939 and Physiol. Rev. 16: 238, 1936; Also J. Am. Med. Assn. 111: 1462, 1938
KING, C. G., AND MENTEN, M. L.: J. Nutrition 10: 129, 1935.
KUGELMASS, I. N.: J. Amer. Med. Assn. 115: 519, 1940.
LEVINE, S. Z., MARPLES, E., AND GORDON, H. H.: J. Clin. Investigation 20: 199, 1941.
LIND, JAMES: Treatise on Scurvy, London, 1757.
LORENZ, A. J., AND ARNOLD, L. J.: Food Research J. 6: 151, 1941.
McCOLLUM, E. V., AND PITZ, W.: J. Biol. Chem. 31: 229, 1917.
PARSONS, H. T.: J. Biol. Chem. 44: 587, 1920 and ibid 59: 97, 1924.
REICHSTEIN, T., GRUSSNER, A., AND OPPENHAUER, R.: Helv. chim. acta. 16: 1019, 1933.
REID, M. E.: Amer. J. Bot. 25: 701, 1938 and Bull. Torrey Bot. Club 69: 204, 1942.
SCARBOROUGH, H.: Biochem. J. 33: 1400, 1939.
SULZBERGER, M. B., AND OSER, B. L.: Proc. Soc. Exptl. B & M. 32: 716, 1935.
SZENT, GYORGYI A., AND HAWORTH, W. N.: Nature 131: 23, 1933.
SZENT, GYORGYI A.: Biochem. J. 22: 1387, 1928; and Biological Oxidations. Williams & Wilkins, 1939.
TILLMANS, J., AND HIRSCH, P., AND W.: Ztschr. Untersuchung Lebensmittel 60: 34, 1930 and 63, 1, 1932
VEDDER, E. B.: Mil. Surgeon 49: 133, 503, 1921.
WAUGH, W. A., AND KING, C. G.: J. Biol. Chem. 97: 325, 1932.
WOLBACH, S. B., AND HOWE, P. R.: Arch. Path. and Lab. Med. 1: 1, 1926
ZILVA, S.: Biochem. J. 16: 42, 1922, ibid 17: 410, 1923; ibid 20: 1625, 1926; See also: Arch. Dis. Childhood 10: 253, 1935

CHAPTER XII

ASKEW, F. A., ET AL.: Proc. Roy. Soc., London, 109: 488, 1932.
BILLS, C. E.: J. Amer. Med. Assn. 110: 2150, 1938. See also Physiol. Rev. 15: 1, 1935.
BROCKMAN, H.: Ztschr. f. physiol. Chem. 241: 104, 1936 and Klin. Wochnschr. 16: 1383, 1937.
CEDER, E. T., AND ZON, L.: Pub. Health Report 52: 1580, 1937.
DOTORSKY, A., AND PLATT, S. S.: J. Amer. Med. Assn. 101: 275, 1933.
DALLDORF, G., AND ROWE, C.: Personal communication, 1937.
GOTHLIN, G. F.: Skandinav. Arch. f. Physiol. 80: 133, 1938
HAMILTON, B., AND SCHWARTZ, C.: Am. J. Dis. Chil. 46: 775, 1933.
HASLEWOOD, G. H. D., AND DRUMMOND, J. C.: Chem. and Industr. 55: 598, 1936.
HESS, A. F., AND WEINSTOCK, M.: J. Biol. Chem. 62: 301, 1924.
HOWLAND, J., AND KRAMER, B.: Am. J. Dis. Chil. 22: 105, 1921.
KAY, H. D.: Physiol. Rev. 12: 384, 1932.
KNAPP, A. A.: J. Amer. Med. Assn. 110: 1993, 1938.
McCOLLUM, E. V.: J. Biol. Chem. 45: 333, 1921 and 51: 42, 1922.
MACDONALD, F. G.: J. Biol. Chem. 114: 115, 1936
McLEAN, F. C., AND HASTINGS, A. B.: Tr. A. Am. Phys. 49: 76, 1934. Also J. Biol. Chem. 107: 337, 1934 and 108: 285, 1935. Am. J. Med. Sci. 189: 601, 1935.

MASSENGALE, O. N., AND NUSSMEIER, M.: J. Biol. Chem. 87: 423, 1930.
MELLANBY, E.: Spec. Report Series No. 61. Med. Res. Council, 1921.
MILAS, N. A., AND ANDERSON, W. L : J. Am. Chem. Soc. 61: 2534, 1939.
NICOLLAYSEN, R., AND JANSEN, J.: Arch. Pediatr. 23: 405, 1939.
PARK, E. A.: J. Amer. Med. Assn. 111: 1179, 1938.
PRESNALL, A. K.: J. Biol. Chem 121: 5, 1937.
ROBISON, R.: Herter Lectures, N. Y. Univ. Press, 1932.
ROSENHEIM AND WEBSTER: Biochem. J. 21: 127, 1927.
SCHENCK, F.: Naturwissensch. 25: 159, 1937.
SHERMAN, H. C., AND PAPPENHEIMER, A. M.: J. Exper. Med. 34: 189, 1921.
SHOHL, A. T.: J. Nutrition 14: 69, 1937. See also J. Amer. Med. Assn 111: 614, 1938.
SIMONS, E. J. H., AND ZUCKER, T. F.: J. Am. Chem Soc. 58: 2655, 1936.
STAVELY, H. E., AND BERGMANN, W.: J. Org. Chem. 1: 567, 1937.
STEENBOCK, H., AND BLACK, A.: J. Biol. Chem 64: 263, 1925
STEENBOCK, H., AND BLACK, A.: J. Biol. Chem. 61: 405, 1924 and 64: 263, 1925.
TANRET, M. C.: Ann. chim. phys., Series 5, 17: 493, 1879.
WADDELL, J.: J. Biol. Chem. 105. 711, 1934.
WINDAUS, A., AND TRAUTMAN, G.: Ztschr. f. physiol. chem. 241: 116, 1936.
WINDAUS, A., LINSERT, O., LUTTRINGHAUS, A., WEIDLICH, G.: Ann. Chem. 492: 226, 1932.
WUNDERLICH, W.: Ztschr. f. physiol. chem. 241: 116, 1936.

CHAPTER XIII

EMMERIE, A , AND ENGEL, C.: Nature 142: 873, 1938.
EVANS, H. M.: J. Amer. Med. Assn. 99: 469, 1932.
EVANS, H. M., AND BISHOP, K. S : Am. J. Physiol. 63: 396, 1922.
FURTER, E. M., AND MEYER, R. E.: Helv. chim acta 22: 240, 1939.
GOETSCH, M , AND PAPPENHEIMER, A M.: J. Exper. Med. 54: 145, 1931. See also J. Nutrition 17· 371, 1939.
HICKMAN, K.: J Biol. Chem. 128: 93, 1939.
KARRER, P.: Helv chim acta 21 239, 1938 and 21: 1161, 1938.
MATTILL, H A : J. Amer. Med. Assn 110: 1831, 1938.
OLCOTT, H S , AND MATTILL, H. A.. J. Biol. Chem. 104: 423, 1934. See also ibid 110: 695, 1935.
SURE, B : Science 59: 19, 1924 and J Biol. Chem. 58: 693, 1924.

CHAPTER XIV

ALMQUIST, H. J., AND STOKSTAD, E L R : J. Biol. Chem. 111: 105, 1935
DAM, H.: Biochem J. 29· 1273, 1935.
DAM, H : Biochem Ztschr. 215. 475, 1929 and 220: 158, 1930. See also Ann. Rev. Biochem. 1940
DAM, H., GEIGER, A , GLAVIND, J , KARRER, W., ROTHSCHILD, E. AND SALOMON, H.: Helv chim. acta 22 310, 1939
MCFARLANE, W. D., GRAHAM, W R , RICHARDSON, F.· Biochem. J. 25: 358, 1931
MCKEE, R W , BINKLEY, S. B., MACCORQUODALE, D. W., THAYER, S. A., AND DOISY, E. A.· J Am. Chem Soc 61. 1295, 1939.
MCKEE, R W , BINKLEY, S. B., THAYER, S. A., MACCORQUODALE, D. W., AND DOISY, E. A. J. Biol. Chem 131: 327, 1939
QUICK, A. J.· J. Amer Med. Assn. *110· 1658, 1938. See also* Am. J. Med. Sci. 190: 501, 1935.

CHAPTER XV

Vitamins and Disease

Aschoff, L.: personal communication.
Barborka, C. J., Foltz, E. E, and Ivy, A C: J. A. M. A. 122: 717, 1943.
Budd, G.: Tweedie's System of Practical Medicine, Philadelphia, 1840.
Burke, B. S., Beal, V. A., Kirkwood, S. B., and Stuart, H. C.: Am. J. Ob. & Gyn. 46: 38, 1943.
Carlson, A. J.: Northwest Medicine 41: 334, 1942.
Clendenning, L.: J. A. M A. 117: 1035, 1941.
Ebbs, J. H., Tisdall, F. F., and Scott, W. A: J. Nutrition 22: 515, 1941.
Elvehjem, A. C.: Chem. Eng. News 21: 853, 1943.
Foster, C., Jones, J. H., Henle, W., and Dorfman, F.: J. Exper. Med. 79: 221, 1944.
Funk, C.: J. Physiol. 43: 395, 1911.
Hess, A. F.: Rickets Including Osteomalacia and Tetany, Philadelphia, Lea and Febiger, 1929.
Hoelzel, F.: Science 95: 251, 1942.
Holmes, C. E., Deobold, H. J, and Herrick, C. A.: Poultry Science 17: 136, 1938.
Hopkins, F. G.: Analyst 31: 385, 1906
Jolliffe, N. (Conditioned Malnutrition), Handbook of Nutrition, Chicago, Am. Med. Ass., 1943
Jolliffe, N., McLester, J S, and Sherman, H. C.: J. A. M. A. 118. 944, 1942.
Kellogg, C. A.: Ann Am. Acad. Pol. & Soc. Sc. 225. 19, 1943.
Lambooy, J. P., and Nasset, E. S: J Nutrition 26: 293, 1943.
Phipard, E. F.: Ann Am Acad. Pol. & Soc. Sc 225: 66, 1943.
Price, W. A.: Nutrition and Physical Degeneration, Hoeber, 1939.
Sebrell, W H. U S Pub Health Rep 58: 803, 1943.
Sherman, H. C.: The Science of Nutrition, New York, Columbia Univ. Press, 1943.
Smith, S. G., Curry, R., and Hawfield, H: Science 98: 520, 1943.
Spicknall, C. G., Fishburn, H. D, and Baum, W. S.: U. S. Pub. Health Rep. 58: 1669, 1943
Spies, T. D.: J. A M A 122: 911, 1943.
Stare, F. J.. Bull. N Y Acad. Med. 20: 237, 1944
van Veen, A. G Ann. Rev Biochemistry, Vol XI, 1942.
Waisman, H A., et al: J Nutrition 26: 205, 1943.
Wells, O. V. (quoted by Cassels, J M., and Hall, F L: Ann. Am. Acad. Pol. & Soc Sc. 225: 106, 1943).

CHAPTER XVI

Vitamin A Deficiency

Abelin, I.. Hoppe Seyler Ztschr f physiol Chem. 217: 109, 1933.
Aberle, S. B. D.. J. Nutrition 7. 445, 1934
Altschule, M. D. Arch Path 20. 845, 1935.
Andervont, H B: Pub Health Rep 54: 2085, 1939.
Aschoff, L., and Koch, W.: Skorbut Eine Pathologischanatomische Studie, Jena, Fischer, 1919
Aykroyd, W. R, and Wright, R. E.: Indian J. M. Res 25: 7, 1937.
Aykroyd, W. R., and Rajagopal, K.: Indian J. M. Res. 24: 419, 1936.
Balo, J., and Ballon, H. C.: Arch. Path. 7: 27, 1929.
Batchelder, E. L.: Am. J. Physiol. 109. 430, 1930

MASSENGALE, O. N., AND NUSSMEIER, M.: J. Biol. Chem. 87: 423, 1930.
MELLANBY, E.: Spec. Report Series No 61. Med. Res. Council, 1921.
MILAS, N. A., AND ANDERSON, W. L.: J. Am. Chem. Soc. 61: 2534, 1939
NICOLLAYSEN, R., AND JANSEN, J.: Arch. Pediatr. 23: 405, 1939.
PARK, E, A.: J. Amer. Med. Assn. 111: 1179, 1938.
PRESNALL, A. K.: J. Biol. Chem. 121: 5, 1937.
ROBISON, R.: Herter Lectures, N. Y. Univ. Press, 1932.
ROSENHEIM AND WEBSTER: Biochem. J. 21: 127, 1927.
SCHENCK, F.: Naturwissensch. 25: 159, 1937.
SHERMAN, H. C., AND PAPPENHEIMER, A. M.: J. Exper. Med. 34: 189, 1921.
SHOHL, A. T.: J. Nutrition 14: 69, 1937. See also J. Amer. Med. Assn. 111: 614, 1938.
SIMONS, E. J. H., AND ZUCKER, T. F.: J. Am. Chem Soc. 58: 2655, 1936.
STAVELY, H. E., AND BERGMANN, W.: J. Org. Chem. 1: 567, 1937.
STEENBOCK, H., AND BLACK, A.: J. Biol. Chem. 64: 263, 1925.
STEENBOCK, H., AND BLACK, A.: J. Biol. Chem. 61: 405, 1924 and 64: 263, 1925.
TANRET, M. C.: Ann chim. phys, Series 5, 17: 493, 1879.
WADDELL, J.: J. Biol. Chem. 105: 711, 1934.
WINDAUS, A., AND TRAUTMAN, G.: Ztschr. f. physiol. chem. 241: 116, 1936.
WINDAUS, A., LINSERT, O., LUTTRINGHAUS, A., WEIDLICH, G.: Ann. Chem. 492: 226, 1932.
WUNDERLICH, W.: Ztschr. f. physiol. chem. 241: 116, 1936.

CHAPTER XIII

EMMERIE, A., AND ENGEL, C.: Nature 142: 873, 1938.
EVANS, H. M.: J. Amer Med. Assn. 99: 469, 1932.
EVANS, H. M., AND BISHOP, K. S.: Am. J. Physiol. 63: 396, 1922.
FURTER, E. M., AND MEYER, R. E.: Helv. chim acta 22: 240, 1939.
GOETSCH, M, AND PAPPENHEIMER, A. M.: J. Exper. Med. 54: 145, 1931. See also J. Nutrition 17: 371, 1939.
HICKMAN, K.: J. Biol. Chem. 128: 93, 1939.
KARRER, P.: Helv. chim. acta 21: 239, 1938 and 21: 1161, 1938.
MATTILL, H A.: J. Amer Med. Assn. 110: 1831, 1938.
OLCOTT, H. S., AND MATTILL, H. A.: J. Biol Chem. 104: 423, 1934. See also ibid 110: 695, 1935.
SURE, B.: Science 59: 19, 1924 and J Biol. Chem. 58: 693, 1924.

CHAPTER XIV

ALMQUIST, H. J, AND STOKSTAD, E. L R.: J. Biol. Chem. 111: 105, 1935.
DAM, H.: Biochem. J 29: 1273, 1935.
DAM, H: Biochem Ztschr. 215: 475, 1929 and 220: 158, 1930. See also Ann. Rev. Biochem. 1940.
DAM, H., GEIGER, A., GLAVIND, J., KARRER, W., ROTHSCHILD, E. AND SALOMON, H.: Helv. chim acta 22 310, 1939.
MCFARLANE, W. D, GRAHAM, W. R., RICHARDSON, F: Biochem. J. 25: 358, 1931.
MCKEE, R. W., BINKLEY, S B., MACCORQUODALE, D. W., THAYER, S. A., AND DOISY, E. A: J Am. Chem Soc. 61: 1295, 1939
MCKEE, R. W., BINKLEY, S. B., THAYER, S A., MACCORQUODALE, D. W., AND DOISY, E. A: J Biol. Chem 131: 327, 1939.
QUICK, A. J.: J. Amer. Med. Assn. 110 1658, 1938. See also Am. J. Med. Sci. 190: 501, 1935.

CHAPTER XV

Vitamins and Disease

ASCHOFF, L.: personal communication.
BARBORKA, C. J., FOLTZ, E. E., AND IVY, A. C.: J. A. M. A. 122: 717, 1943.
BUDD, G.: Tweedie's System of Practical Medicine, Philadelphia, 1840.
BURKE, B. S., BEAL, V. A., KIRKWOOD, S. B., AND STUART, H. C.: Am. J. Ob. & Gyn. 46: 38, 1943.
CARLSON, A. J.: Northwest Medicine 41: 334, 1942.
CLENDENNING, L.: J. A. M. A. 117: 1035, 1941.
EBBS, J. H., TISDALL, F. F., AND SCOTT, W. A.: J. Nutrition 22: 515, 1941.
ELVEHJEM, A. C.: Chem. Eng. News 21: 853, 1943.
FOSTER, C., JONES, J. H., HENLE, W., AND DORFMAN, F.: J. Exper. Med. 79: 221, 1944.
FUNK, C.: J. Physiol. 43: 395, 1911.
HESS, A. F.: Rickets Including Osteomalacia and Tetany, Philadelphia, Lea and Febiger, 1929.
HOELZEL, F.: Science 95: 251, 1942.
HOLMES, C. E., DEOBOLD, H. J., AND HERRICK, C. A.: Poultry Science 17: 136, 1938.
HOPKINS, F. G.: Analyst 31: 385, 1906.
JOLLIFFE, N. (Conditioned Malnutrition), Handbook of Nutrition, Chicago, Am. Med. Ass., 1943.
JOLLIFFE, N., MCLESTER, J. S., AND SHERMAN, H. C.: J. A. M. A. 118: 944, 1942.
KELLOGG, C. A.: Ann. Am. Acad. Pol. & Soc. Sc. 225: 19, 1943.
LAMBOOY, J. P., AND NASSET, E. S.: J. Nutrition 26: 293, 1943.
PHIPARD, E. F.: Ann. Am. Acad. Pol. & Soc. Sc. 225: 66, 1943.
PRICE, W. A.: Nutrition and Physical Degeneration, Hoeber, 1939.
SEBRELL, W. H.: U. S. Pub. Health Rep. 58: 803, 1943.
SHERMAN, H. C.: The Science of Nutrition, New York, Columbia Univ. Press, 1943.
SMITH, S. G., CURRY, R., AND HAWFIELD, H.: Science 98: 520, 1943.
SPICKNALL, C. G., FISHBURN, H. D., AND BAUM, W. S.: U. S. Pub. Health Rep. 58: 1669, 1943.
SPIES, T. D.: J. A. M. A. 122: 911, 1943.
STARE, F. J.: Bull. N. Y. Acad. Med. 20: 237, 1944.
VAN VEEN, A. G.: Ann. Rev. Biochemistry, Vol. XI, 1942.
WAISMAN, H. A., ET AL.: J. Nutrition 26: 205, 1943.
WELLS, O. V. (quoted by CASSELS, J. M., AND HALL, F. L.: Ann. Am. Acad. Pol. & Soc. Sc. 225: 106, 1943).

CHAPTER XVI

Vitamin A Deficiency

ABELIN, I.: Hoppe Seyler Ztschr. f. physiol. Chem. 217: 109, 1933.
ABERLE, S. B. D.: J. Nutrition 7: 445, 1934.
ALTSCHULE, M. D.: Arch. Path. 20: 845, 1935.
ANDERVONT, H. B.: Pub. Health Rep. 54: 2085, 1939.
ASCHOFF, L., AND KOCH, W.: Skorbut. Eine Pathologischanatomische Studie, Jena, Fischer, 1919.
AYKROYD, W. R., AND WRIGHT, R. E.: Indian J. M. Res. 25: 7, 1937.
AYKROYD, W. R., AND RAJAGOPAL, K.: Indian J. M. Res. 24: 419, 1936.
BALO, J., AND BALLON, H. C.: Arch. Path. 7: 27, 1929.
BATCHELDER, E. L.: Am. J. Physiol. 109: 430, 1930.

Birch-Hirschfeld, A.: Arch. f. Ophth. 92: 273, 1916.
Blackfan, K. D., and Wolbach, [redacted]. B.: J. Pediat. [redacted]: 679, 1933.
Blegvad, O.: Am. J. Ophth. 7: 89, 1924.
Bloch, C. E.: Am. J. Dis. Child. 28: 659, 1924.
Braunschweig: Münch. med. Wchnschr. 9, 1915.
Brazer, J. G., and Curtis, A. C.: Arch. Int Med. 65: 90, 1940.
Breese, B. B., Jr, and McCoord, A. B.: J. Pediat. 15: 183, 1939.
Browman, L. G.: Am. J. Physiol. 125: 335, 1939.
Cannon, M. D.: Proc. Soc. Exper. Biol. & Med. 44: 129, 1940.
Carleton, A., and Steven, A: Arch. Derm. & Syph. 48: 143, 1943.
Chalier, J., and Jeune, M.: *Compt. rend. Soc. de biol. 129. 604, 1938.*
Choun, B.: Am. J. Dis. Child. 57: 489, 1939.
Clayton, C. C., and Baumann, C. A.: J. Nutrition 27: 155, 1944.
Codvelle, F.: Rev. de méd, Paris 19: 347, 1938.
Collazo, J. A., and Rodriguez, J. S.: Klin. Wchnschr. 12: 1732 and 1768, 1933.
Cornell, N W. (personal communication).
Cornil, L., Chevallier, A., and Paillas, J. E.: Ann. d'anat. Path. 16: 74, 1939.
Creech, G. T., and Seibold, H. R.: Am. J. Vet. Res. 4: 353, 1943.
Dalldorf, G.: Bull New York Acad. Med. 14: 635, 1938.
Drigalski, W. v.: Klin. Wchnschr. 12: 308, 1933.
Drigalski, W. v: Ernährung 5: 181, 1940.
Duncan, D.: Arch. Neurol. & Psychiat. 25. 327, 1941.
Eriksen, B., and Höygaard, A.: Klin Wchnschr. 20: 200, 1941.
Erspamer, V.: Virchows Arch. f. path. Anat. 302: 766, 1938.
Evans, H. M., and Bishop, K. S.: Anat. Rec. 23: 17, 1922.
Eveleth, D. F., and Biester, H. E.: Am. J. Path. 13: 257, 1937.
Fisher, O. E.: Brit. M. J. 2: 944, 1938.
Forest and Wolff: J. de méd. de Paris 52: 302, 1932.
Frandsen, H.: Acta Ophth., suppl. 4, 1935.
Fraser and Cameron (quoted by McCarrison).
Frazier, C. N., and Hu, C.: Arch. Int. Med. 48: 507, 1931. Also Arch. Derm. & Syph. 33. 825, 1936.
Frazier, C. N., and Li, H C.: Chinese M. J. 54: 301, 1938.
Fridericia, L. S. et al.. Am. J. Cancer 39: 61, 1940.
Goerner, A., and Goerner, M. M.: J. Biol. Chem. 123: 57, 1938. J. Nutrition 18: 441, 1939.
Goldblatt, H, and Benischek, M J. Exper. Med. 46: 699, 1927.
Greaves, J D, and Schmidt, W.: J. Biol. Chem. 105: 31, 1934. Am. J. Physiol. 116: 456, 1936.
Guilbert, H. R., Howard, and Hart, G. E: J. Nutrition 19: 91, 1940.
Haas, J H., and Meulemans, O.. Lancet 1: 1110, 1938.
Haig, C., and Patek, A. J, Jr J. Clinic. Invest. 21: 309, 1942.
Hale, F Texas State J. Med. 33: 228: 1937.
Haythorn, S. R. J. M. Res 26: 523, 1912.
Hecht, S: Ann. Rev. Biochem. Vol. 11, 1942.
Hecht, S., and Mandelbaum, J.: J. A. M A. 112: 1910, 1939.
Herbert (quoted by Bloch)
Hickman, K., Harris, P. L., and Woodside, M. R.: Nature 150: [redacted], 1942.
Higgins, C. C.: J. Urol. [redacted]. 157, 1933.

Ralli, E. P., Pariente, A. C., Brandaleone, H., and Davidson, S.: J. A. M. A. 106: 1975, 1936.
Schneider, E.: Vorträge aud d. prakt. Chirurgie 17: [illegible], 1937.
Schneider, E., and Weigand, H.: Klin. Wchnschr. 16: 441, 1937.
Schour, I., Smith, M. C., and Hoffman, M. M.: Proc. Soc. Exper. Biol. & Med. 39: 447, 1938.
Schulze, E., and Hundhausen, G.: Arch. f. exper. Path. u. Pharmakol. 192: 43, 1939.
Sebrell, W. N.: Handbook of Nutrition. Chicago, Am. Med. Ass., 1943.
Seifried, O.: J. Exper. Med. 52: 519, 1930.
Setterfield, H. E., and Sutton, T. S.: J. Nutrition 9: 645, 1935.
Sharpless, G. H.: J. Nutrition 19: 31, 1940
Sherman, H. C., and MacLeod, F. L.: J. Am. Chem. Soc. 47: 1658, 1925.
Sherman, W. C.: J. Nutrition 22: 153, 1941.
Sherwood, T. C., Depp, O. R, Birge, G. P, and Dotson, H. B.: J. Nutrition 14: 481, 1937.
Silberberg, M, and Silberberg, R.: Arch. Path. 28: 340, 1939.
Simon, R. S.: J. Bone & Joint Surg. 24: 681, 1942.
Steffens, L. F., Bair, H. L., and Sheard, C.: Proc. Staff Meet., Mayo Clin. 14: 698, 1939.
Steiner, M., Zuger, B., and Kramer, B.: Arch. Path. 27: 104, 1939.
Stiebeling, H. K.: Handbook of Nutrition. Chicago, Am. Med. Ass., 1943.
Sullivan, M., and Evans, V. J.: J. Nutrition 25: 319, 1943.
Sweet, L. K., and K'ang, H. J. Am. J. Dis. Child 50: 699, 1935.
Thatcher, H. S., and Sure, B.: Arch. Path. 13: 756, 1932.
Tilden, E. B., and Miller, E. G.: J. Nutrition 3: 121, 1930.
Tricoire (quoted by Codvelle).
Vaillant, C., and Gillis, L.: Lancet 236: 149, 1939.
Van Leersum, E. C.: J. Biol. Chem. 76: 137, 1928.
Vedder, E. B., and Rosenberg, C.: J. Nutrition 16: 57, 1938.
Wagner, K. H., Hoppe-Seyler, Z. 264: 153, 1940.
Wald, G., Jeghers, H., and Arminio, J.: Am. J. Physiol. 123: 723, 1938.
Warren, S.: The Pathology of Diabetes Mellitus, Philadelphia, Lea & Febiger, 1938 (2nd ed.).
Wecker: Bull. Acad de méd. 36: 4, 1916.
Wendt, H.: Klin Wchnschr. 16: 1175, 1937 (saturation test). Also Münch. Med. Wchnschr. 82: 1679, 1935 (thyroxin).
Wilbur, D. L., and Eusterman, G. B. J A. M. A. 102: 364, 1934.
Wilson, J. R., and DuBois, R. O. Am. J. Dis. Child. 26: 431, 1923.
Wolbach, S B. The Vitamins. A Symposium Chicago, Am. Med. Ass, 1939.
Wolbach, S. B, and Bessey, O. A.: Science 91 · 599, 1940.
Wolbach, S. B, and Bessey, O. A: Physiol. Rev. 22: 233, 1942.
Wolbach, S. B., and Howe, P. E.: J. Exper. Med. 42: 753, 1925.
Youmans, J. B., and Corlette, M. B.: Am. J. M. Sc 195: 644, 1938 (skin lesions), also J. Lab & Clin. Med 23: 663, 1938 (conjunctival smears)
Yudkin, A. M: Bull. New York Acad. Med. 15: 406, 1939.
Zimmerman, H M J. Exper. Med. 57: 215, 1933.
Zimmerman, H. M, and Cowgill, G R · J Nutrition 11: 411, 1936.
Zuckerman, S, and Parkes, A. S: J. Anat 70: 323, 1936.

CHAPTER XVII

Thiamine Deficiency

ALEXANDER, L.: Am. J. Path. 16: 61, 1940.
ARNOLD, A., AND ELVEHJEM, C. A.: Am. J. Physiol. 126: 289, 1939.
ASHBURN, L. L., AND LOWRY, J. V.: Arch. Path. 37: 27, 1944.
AYKROYD, W. R., AND KRISHNAN, B. G.: Indian J. Med. Res. 29: 551, 1941.
BAKER, A. Z., AND WRIGHT, M. D.: Proc. Roy. Soc. Med. 29: 1145, 1936.
BÄLZ, E.: Mitt. d. dtsch. Ges. f. Natur-u. Volkerkünde Ostasiens, II. 27, 1882.
BEAZELL, J. M., AND IVY, A. C.: Arch. Path. 22: 213, 1936.
BRAY, G. W.: Tr. Roy. Soc. Trop. Med. & Hyg. 22: 9, 1928.
BUEDING, E., AND WORTIS, H.: J. Biol. Chem. 133: 585, 1940.
CAMPBELL, A. C. P., AND BIGGART, J. H.: J. Path. & Bact. 48: 245, 1940.
CHURCH (quoted by Swank).
COWGILL, G. R.: The Vitamin B₁ Requirements of man. Yale Univ. Press, 1934.
COWGILL, G. R., DEUEL, H. J., PLUMMER, N., AND MESSER, F. C.: Am. J. Physiol. 77: 389, 1926.
COWGILL, G. R., AND GILMAN, A.: Arch. Int. Med. 53: 58, 1934.
DALLDORF, G., AND KELLOGG, M.: J. Exper. Med. 56: 391, 1932.
DANN, M., AND COWGILL, G. R.: Arch. Int. Med. 62: 137, 1938.
DRUMMOND, J. C., BAKER, A. Z., WRIGHT, M. D., MARRIAN, P. M., AND SINGER, E. M.: J. Hyg. 38: 356, 1938.
DRUMMOND, J. C., AND WILBRAHAM, A.: The Englishman's Food. London, Jonathan Cape, 1939.
DUSTIN, C., WEYLER, H., AND ROBERTS, C. P.: New Eng. J. Med. 220: 15, 1939.
ELSOM AND MACHELLA: Am. J. Med. Sc. 202: 602, 1941.
ENDER, F., AND HELGEBOSTAD, F.: Skandinavisk Veterinär-Tidskrift 29: 1232, 1939.
EPPINGER, H.: Die Seröse Entzündung. Vienna, Springer, 1935.
EVANS, H. M., LEPKOVSKY, F., AND MURPHY, E. A.: J. Biol. Chem. 107: 421, 1934.
EVANS, H. M., AND BISHOP, K. S.: J. Metabolic Res. 1: 356, 1932.
EVANS, C. A., CARLSON, W. E., AND GREEN, R. G.: Am. J. Path. 18: 79, 1942.
FEHILY, L.: J. Trop. Med. & Hyg. 44: 21, 1941.
FRAZER, H., AND STANTON, A. T.: J. Trop. Med. 14: 333, 349 and 366, 1911.
FRIDERICIA, L. S.: Skand. Arch. Physiol. 2: 55, 1926.
FUKUI (quoted by Shimazono).
FUNK, C.: J. Physiol. 43: 395, 1911.
GOODHART, R.: J. Biol. Chem. 135: 77, 1940.
GOODHART, R., AND SINCLAIR, H. M.: J. Biol. Chem. 132: 11, 1940.
GREEN, R. G., AND EVANS, C. A.: Science 92: 154, 1940.
HARRIS, L. J.: Lancet 1: 886, 1936.
HASHIMOTO: Am. Heart J. 13: 580, 1937.
HECHT, G., AND WEESE, H.: Klin. Wchnschr. 16: 414, 1937.
HONDA: Pub. med. Fac. inp. Univ. Tokyo 11: 319, 1914.
HOWE, P. E.: Ann. Am. Acad. Pol. & Soc. Sc. 225: 72, 1943.
HOWES, E. L., AND VIVIER, P. J.: Am. J. Path. 12: 689, 1936.
JOLLIFFE, N.: Int. Clinics 47, 1938.
JOLLIFFE, N., BOWMAN, K. M., ROSENBLUM, L. A., AND FEIN, H. D.: J. A. M. A. 114: 307, 1940.
KEYS, A., HENSCHEL, A. F., MICKELSEN, O., AND BROZEK, J. M.: J. Nutrition 26: 399, 1943.

Day, P. L.: Am. J. Pub. Health 24: 603, 1934.
Day, P. L., and Darby, W. J.: J. Nutrition 12: 387, 1936.
Day, P. L., Darby, W. J., and Cosgrove, K. W.: J. Nutrition 15: 83, 1938 (flavin). Ibid. 15: 1, 1938 (arrest by flavin).
Eckardt, R. E., and Johnson, L. V.: Arch. Ophth. 21: 315, 1939.
Engel, R. W., and Phillips, P. H.: Proc. Soc. Exper. Biol. & Med. 40: 597, 1939.
Engel, R. W., Phillips, P. H., and Halpin, J. G.: Poultry Sc. 19: 135, 1940.
Goldberger, J., and Lillie, R. D.: U. S. Pub. Health Rep. 41: 1025, 1926.
György, P.: Biochem. J. 29: 741, 1935.
Gyorgy, P.: Proc. Soc. Exper. Biol. & Med. 38: 383, 1938.
György, P., Robscheit-Robbins, F. S., and Whipple, G. H.: Am. J. Physiol. 122: 154, 1938.
Hou, H. C.: Chinese Med. J. 59: 314, 1941.
Jolliffe, N., Fein, H. D., and Rosenblum, L. A.: New England J. Med. 221: 921, 1939.
Keys, A. et al.: J. Nutrition 27: 165, 1944.
Kruse, H. D, Sydenstricker, V. P., Sebrell, W. H., and Cleckley, H. M.: U. S. Pub. Health Rep. 55: 157, 1940
Landor, J. V., and Pallister, R. A.: Tr. Roy. Soc. Trop. Med. & Hyg. 29: 121, 1935.
Lepkovsky, S., and Jukes, T. H.: J. Nutrition 12: 515, 1938.
Manson-Bahr, P.: Trans Roy. Soc. Trop. Med. & Hyg. 34: 347, 1941.
Métivier, V. M.: Am J. Ophthalmol. 24: 1265, 1941.
Mitchell, H. S., and Cook, G. M.: Proc Soc. Exper. Biol. & Med. 39: 325, 1938.
Oden, J. W., Oden, L. H., and Sebrell, W. H.: U. S. Pub. Health Rep. 54: 790, 1939.
Phillips, P. H., and Engel, R. W.: J. Nutrition 16: 451, 1938 (chick path.).
Pock-Steen, P. H.: Aknephaskopia, Geneesk. tijdschr v. Nederl.-Indië 78: 1986, 1939.
Richardson, L. R., and Hogan, A. G.: Mississippi Agric. Exper. State Res. Bull. 241, 1936.
Sebrell, W. H.: U. S Pub. Health Rep. 44: 2697, 1926 (dog).
Sebrell, W. H., and Butler, R. E.: U. S. Pub Health Rep. 53: 2288, 1938. Ibid. 54: 2121, 1939 (ariboflavinosis).
Sebrell, W. H., and Onstott, R. H.: U. S. Pub. Health Rep. 53: 83, 1938 (canine aribofl.).
Sebrell, W. H., et al.: U. S. Pub. Health Rep. 53: 2282, 1938.
Sherman, H. C.: The Science of Nutrition, Columbia Univ Press, 1943.
Smith, S. G., and Martin, D. W.: Proc. Soc. Exper. Biol. & Med. 43: 660, 1940.
Spies, T. D., Bean, W. B., and Ashe, W. F.: J A. M. A. 112: 2414, 1939.
Street, H. R., and Cowgill, G. R.: Am. J. Physiol. 125: 323, 1939.
Sydenstricker, V. P., Geeslin, L. E, Templeton, C. M, and Weaver, J. W.: J. A. M. A. 113: 1697, 1939.
Sydenstricker, V. P., Sebrell, W. H., Cleckley, H. M., and Kruse, H. D.: J. A. M. A. 114: 2437, 1940
Zimmerman, H. M., Cowgill, G R., and Fox, J. C: Arch. Neurol. & Psychiat. 37: 286, 1937.

CHAPTER XIX

Niacin Deficiency

Beckh, W., Ellinger, P., and Spies, T. D.. *Quart J Med. 6: 305, 1937.*
Bing, J., and Broager, B.: Ugeskr. f. laegar 100. 1131, 1938.

Birch, T. W., Chick, H., and Martin, C. J.: Biochem. J. 31: 2065, 1937.
Blankenhorn, M. A.: Ann. Int. Med. 11: 823, 1937.
Bogart, C. N.: J. A. M. A 111: 613, 1938.
Boggs, T. R., and Padget, F.: Bull. Johns Hopkins Hosp. 50: 21, 1932.
Castellani, A. J. Trop Med 41. 291, 1938
Cheldelin, V. H., and Williams, R R.: J. Nutrition 26: 417, 1943.
Chittenden, R H., and Underhill, F. R.: Am. J. Physiol. 44: 13, 1917.
Clark, A.: J. Trop Med 41: 143, 1938
Cleckley, H. M, Sydenstricker, V. P., and Geeslin, L E.: J. A. M. A. 112: 2107, 1939.
Crane-Lillie, M., and Rhoads, C. P: Arch. Path. 18: 459, 1934.
Day, P. L., Langston, W. C., and Darby, W. J.: Proc. Soc. Exper. Biol. & Med. 38: 860, 1938.
Deeks, W. E.: South. M. J 15. 891, 1922.
Denton, J.: Am. J. Trop. Med 5 173, 1925 (human pellagra). Am. J. Path. 4: 341, 1928 (black tongue).
Drummond, J. C.: Science and Nutrition. London, Bacharach, Watts & Co., 1938.
Fitzgerald, G. H.. Indian M Gaz 67 556, 1932.
Flinker, R.: Arch. f. Verdauungskr 57: 282, 1935.
Fouts, P J., Helmer, O. M, Lepkovsky, S., and Jukes, T. H.: Proc. Soc. Exper. Biol & Med. 37: 405, 1937
Frostig, J. P, and Spies, T D: J Am Med Sc. 199. 268, 1940
Funk, C.: Die Vitamine, etc Wiesbaden, Bergmann, 1914.
Goldberger, J., et al. Bull Hyg. Lab 153, 120 U. S Pub. Health Rep 31: 3159, 1916.
Goldberger, J., and Wheeler, G A Bull No 120, Hyg. Lab.
Gray: Am. J. Insanity 223, 1864
Greene, J. A.: Am. J M Sc 195 618, 1938
Gurin, S, and Eddy, W H J Exper. Med 54 421, 1931.
Handler, P.: Proc Soc Exper Biol & Med, 52: 263, 1943.
Hansen-Pruss, O C.. New England J Med. 218 1050, 1938.
Harris, L. J.: Biochem J 31. 1414, 1937
Helmer, O. M., and Fouts, P J J Nutrition 16. 271, 1938.
Herzenberg, H.. Beitr z path Anat u z allg. Path. 96: 97, 1935.
Howe, P. E: Ann Am Acad. Pol & Soc Sc. 225 72, 1943.
Jobling, J. W., and Arnold, L J A. M A 80 365, 1923.
Jolliffe, N., Bowman, K. M, Rosenblum, L. A, and Fein, H. D.: J. A. M. A. 114: 307, 1940.
Kirkland, O.: Int J Orthodontia, Oral Surg and Radiography 22: 1172, 1936.
Kooser, J. H, and Blankenhorn, M A J. A M. A 112: 2581, 1939.
Lambert (see report of Chittenden and Underhill)
Landor, J. V., and Pallister, R. A Tr Roy. Soc Trop Med. & Hyg 29: 121, 1935.
Lewy, F. H., Himwich, H. E, Frostig, J. P, and Spies, T. D.: Science 90: 141, 1939.
Li
Lo
Lu
M.
M.
Manson-Bahr, P., and Ransford, O N.. Lancet 2: 426, 1938.
Margolis, L. H., Margolis, G., and Smith, S. G.: J. Nutrition 17: 63, 1939.
Meulengracht, E.: Am. J. M. Sc. 197. 201, 1939.

MOORE, D. F.: J. Trop. Med. 42: 109, 1939.
MOORE, R. A., SPIES, T. D., AND COOPER, Z. K.: Arch. Derm. & Syph. 46: 100, 1942.
MUSSER, J. H.: *Mississippi Doctor* II: 7, 1939.
NEUSSER: Wien. Med. Wchnschr. 131, 1887.
ORTON, S. T., AND BENDER, L.: Bull. Neurol. Inst. New York, 1: 506, 1931.
PEARSON, P. B., SCHMIDT, H., AND MACKAY, A. K.: Proc. Soc. Exper. Biol. & Med. 40: 423, 1939.
PETRI, S., NORGAARD, F., AND BANDIER, E.: Acta Med. Scandinav. 98: 113, 1938.
PETRI, S., NORGAARD, F., AND BING, J.: Am. J. M. Sc. 145: 717, 1938.
RHOADS, C. P., AND MILLER, D. K.: J. Exper. Med. 58: 585, 1933
ROUSENTOUL, M. A.: Acta dermat.-venereol. 15: 493, 1934.
RUFFIN, J. M., AND SMITH, D. T.: South. M. J. 32: 41, 1939.
RUFFIN, J. M., AND SMITH, D. T.: Clinical Pellagra. By Seale Harris, C. V. Mosby Co., 1941.
SALMON, W. D., GUERRANT, N. B., AND HAYS, I. M.: J. Infect. Dis. 43: 426, 1928.
SEARCY, G. H.: Trans. Med. Ass. Alabama 387, 1907.
SEBRELL, W. H., AND BUTLER, R. E.: J. A. M. A. 111: 2286, 1938.
SMITH, C. E., AND STEVENS, I. M.: Am. J. Hyg. 27: 590, 1938.
SMITH, D. T., PERSONS, E. L., AND HARVEY, H. I.: J. Nutrition 14. 373, 1937.
SNYDER, J. R.: Am. J. Dis. Child 4: 172, 1912.
SPIES, T. D.: J. A. M. A. 105: 1028, 1935 (dietary treatment).
SPIES, T. D., BEAN, W. B., AND STONE, R. E.: J. A. M. A. 111: 584, 1938.
SPIES, T. D., AND CHINN, A. B.: J. Clin. Investigation 14: 941, 1935.
SPIES, T. D., CHINN, A. B., AND MCLESTER, J. B.: J. A. M. A. 108: 853, 1937.
SPIES, T. D., COOPER, C., AND BLANKENHORN, M. A.: J. A. M. A. 110: 622, 1938.
SPIES, T. D., GRANT, J. M., STONE, R. E., AND MCLESTER, J. B.: South. M. J. 31: 1231, 1938.
SPIES, T. D., SASAKI, Y., AND CROSS, E.: South. M. J. 31: 483, 1938.
SYDENSTRICKER, V. P., ARMSTRONG, E. S., DERRICK, C. J., AND KEMP, P. S.: Am. J. M. Sc. 192: 1, 1936.
SYDENSTRICKER, V. P.: Clinical Pellagra. By Seale Harris, C. V. Mosby Co. 1940.
TOPPING, N. H., AND FRASER, H. F.: *U. S. Pub. Health Rep. 54: 416, 1939.*
TSCHERKES, L. A., LITVACK, J. I., AND KOROVITZKY, L. K.: Acta med Skand. 87: 459, 1936.
TUCKER, B. R.: South M. J. 28: 603, 1935.
TUCKER, B. R.: South. Med. & Surg. 97: 391, 1935 (etiol.). South. M. J. 28: 603, 1935 (path.).
TYLER, quoted by Harris, S., Clinical Pellagra, C. V. Mosby Co., 194.
VILTER, S. P., SPIES, T. D., AND MATHEWS, A. P.: J. Biol. Chem. 125: 85, 1938.
VOEGTLIN, C., AND HARRIES, W. H.. Bull. Hyg. Lab. 116, 1920.
VOEGTLIN, C., NEILL, M. H., AND HUNTER, A.: Bull. Hyg. Lab. 116, 1920.
WATSON, C. J. Proc. Soc. Exper. Biol. & Med. 41: 591, 1939.
WEISS, S., AND WILKINS, R. W.: Ann. Int Med. 11: 104, 1937.
WILLIAMS, C. D.: Arch Dis. Childhood 8: 423, 1933.
WILSON, K.: Proc Roy. Soc. Med. 7: 31, 1914
WILSON, R. M.: Chinese M. J. 47: 223, 1933 (pellagroid syndrome).
WRIGHT, E. J.: Brit. Med. J 2: 707, 1936.
ZIMMERMAN, H. M., AND BURACK, E.: J. Exper. Med. 59: 21, 1934.

CHAPTER XX

Vitamin B Complex

György, P.: J. Am. Chem. Soc. 60: 983, 1938.
Miller, D. K., and Rhoads, C. P.: J. Exper. Med. 61: 173, 1935.
Wills, L., Clutterbuck, P. W., and Evans, B. D. F.: Biochem. J. 31: 2136, 1937.
Wintrobe, M. M.: Am. J. M. Sc. 197: 286, 1939. Am. J. Physiol. 126: 375, 1939.

Pyridoxine

Antopol, W., and Unna, K.: Proc. Soc. Exper. Biol. & Med. 42: 126, 1939.
Antopol, W., and Schotland, C. E.: J. A. M. A. 114: 1058, 1940.
Chick, H., Macrae, T. F., Martin, A. J. P., and Martin, C. J.: Biochem. J. 32: 2207, 1938.
Dimick, M. K., and Schreffler, C. B.: J. Nutrition 17: 23, 1939.
Flexner, J., and Chassin, M. R.: J. Clin. Invest. 20: 313, 1941.
György, P.: J. Nutrition 16: 69, 1938.
Harris, S. A., and Folkers, S. K.: Science 89: 347, 1939.
Jolliffe, N.: Minn. Med. 23: 542, 1940.
Kark, R., Lozner, E. L., and Meiklejohn, A. P.: Proc. Soc. Exper. Biol. & Med. 43: 97, 1940.
Kuhn, R., Westphal, K., Wendt, G., and Westphal, O.: Naturwissenschaften 27: 469, 1939.
Machella, T. E.: Am. J. Med. Sc. 203: 114, 1942.
Morgan, A. F.: Science 93: 261, 1941.
Schneider, H., Steenbock, H., and Platz, B. R.: J. Biol. Chem. 132: 539, 1930.
Smith, S. G., and Martin, D. W.: Proc. Soc. Exper. Med. & Biol. 43: 660, 1940.
Spies, T. D., Bean, W. B., and Ashe, W. F.: J. A. M. A. 112: 2414, 1939.
Spies, T., Hightower, D. P., and Hubbard, L. H.: J. A. M. A. 115: 292, 1940.
Spies, T., Ladisch, R. K., and Bean, W. B.: J. A. M. A. 115: 839, 1940.
Street, H. R., Cowgill, G. R., and Zimmerman, H. M.: J. Nutrition 21: 275, 1941.
Thomas, R. M., Mylon, E., and Winternitz, M. C.: Yale J. Biol. Med. 12: 345, 1940.
Vilter, S. P., Spies, T. D., and Mathews, A. P.: J. Biol. Chem. 125: 85, 1938.
Wintrobe, M. M., Follis, R. H., Jr., and others: Bull. Johns Hopkins Hosp. 72: 1, 1943.

Pantothenic Acid

Ashburn, L. L.: U. S. Pub. Health Rep. 55: 1337, 1940.
Brandaleone, H., Main, E., and Steele, M.: Proc. Soc. Exper. Biol. & Med. 53: 47, 1943.
Daft, F. S., and Sebrell, W. H.: U. S. Pub. Health Rep. 54: 2247, 1939.
Daft, F. S., Sebrell, W. H., Babcock, S. H., Jr., and Jukes, T. H.: U. S. Pub. Health Rep. 55: 1333, 1940.
Figge, F. H. J., and Soloman, K.: J. Lab. & Clin. Med. 27: 1495, 1942.
Gross, P., Harvalik, Z., and Runne, E.: J. Invest. Dermatol. 4: 385, 1941.
György, P., and Goldblatt, H.: J. Exper. Med. 70: 185, 1939.
György, P., Goldblatt, H., Miller, F. R., and Fulton, R. P.: J. Exper. Med. 66: 579, 1937.
Martin, G. J., and Ansbacher, S.: Proc. Soc. Exper. Biol. & Med. 48: 118, 1941.

MOORE, D. F.: J. Trop. Med. 42: 109, 1939.
MOORE, R. A., SPIES, T. D., AND COOPER, Z. K.: Arch. Derm. & Syph. 46: 100, 1942.
MUSSER, J. H.: Mississippi Doctor 16: 7, 1939.
NEUSSER: Wien. Med. Wchnschr. 131, 1887.
ORTON, S. T., AND BENDER, L.: Bull. Neurol. Inst. New York, 1: 506, 1931.
PEARSON, P. B., SCHMIDT, H., AND MACKAY, A. K.: Proc. Soc. Exper. Biol. & Med. 40: 423, 1939.
PETRI, S., NORGAARD, F., AND BANDIER, E.: Acta Med. Scandinav. 98: 113, 1938.
PETRI, S., NORGAARD, F., AND BING, J.: Am. J. M. Sc. 145: 717, 1938.
RHOADS, C. P., AND MILLER, D. K.: J. Exper. Med. 58: 585, 1933.
ROUSENTOUL, M. A.: Acta dermat.-venereol. 15: 493, 1934.
RUFFIN, J. M., AND SMITH, D. T.: South. M. J. 32: 41, 1939.
RUFFIN, J. M., AND SMITH, D. T.: Clinical Pellagra. By Seale Harris, C. V. Mosby Co., 1941.
SALMON, W. D., GUERRANT, N. B., AND HAYS, I. M.: J. Infect. Dis. 43: 426, 1928.
SEARCY, G. H.: Trans. Med. Ass. Alabama 387, 1907.
SEBRELL, W. H., AND BUTLER, R. E.: J. A. M. A. 111: 2286, 1938.
SMITH, C. E., AND STEVENS, I. M.: Am. J. Hyg. 27: 590, 1938.
SMITH, D. T., PERSONS, E. L., AND HARVEY, H. I.: J. Nutrition 14: 373, 1937.
SNYDER, J. R.: Am. J. Dis. Child 4: 172, 1912.
SPIES, T. D.: J. A. M. A. 105: 1028, 1935 (dietary treatment).
SPIES, T. D., BEAN, W. B., AND STONE, R. E.: J. A. M. A. 111: 584, 1938.
SPIES, T. D., AND CHINN, A. B.: J. Clin. Investigation 14: 941, 1935.
SPIES, T. D., CHINN, A. B., AND MCLESTER, J. B.: J. A. M. A. 108: 853, 1937.
SPIES, T. D., COOPER, C., AND BLANKENHORN, M. A.: J. A. M. A. 110: 622, 1938.
SPIES, T. D., GRANT, J. M., STONE, R. E., AND MCLESTER, J. B.: South. M. J. 31: 1231, 1938.
SPIES, T. D., SASAKI, Y., AND CROSS, E.: South. M. J. 31: 483, 1938.
SYDENSTRICKER, V. P., ARMSTRONG, E. S., DERRICK, C. J., AND KEMP, P. S.: Am. J. M. Sc. 192: 1, 1936.
SYDENSTRICKER, V. P.: Clinical Pellagra. By Seale Harris, C. V. Mosby Co. 1940.
TOPPING, N. H., AND FRASER, H. F.: U. S. Pub. Health Rep. 54: 416, 1939.
TSCHERKES, L. A., LITVACK, J. I., AND KOROVITZKY, L. K.: Acta med. Skand. 87: 459, 1936.
TUCKER, B. R.: South. M. J. 28: 603, 1935.
TUCKER, B. R.: South. Med. & Surg. 97: 391, 1935 (etiol.). South. M. J. 28: 603, 1935 (path.).
TYLER, quoted by Harris, S., *Clinical Pellagra*, C. V. Mosby Co., 194.
VILTER, S. P., SPIES, T. D., AND MATHEWS, A. P.: J. Biol. Chem. 125: 85, 1938.
VOEGTLIN, C., AND HARRIES, R. H.: Bull. Hyg. Lab. 116, 1920.
VOEGTLIN, C., NEILL, M. H., AND HUNTER, A.: Bull. Hyg. Lab. 116, 1920.
WATSON, C. J.: Proc. Soc. Exper. Biol. & Med. 41: 591, 1939.
WEISS, S., AND WILKINS, R. W.: Ann. Int. Med. 11: 104, 1937.
WILLIAMS, C. D.: Arch. Dis. Childhood 8: 423, 1933
WILSON, K.: Proc. Roy. Soc. Med. 7: 31, 1914.
WILSON, R. M.: Chinese M. J. 47: 223, 1933 (pellagroid syndrome).
WRIGHT, E. J.: Brit. Med. J. 2: 707, 1936.
ZIMMERMAN, H. M., AND BURACK, E.: J. Exper. Med. 59: 21, 1934

CHAPTER XX

Vitamin B Complex

György, P : J. Am Chem. Soc. 60. 983, 1938.
Miller, D. K., and Rhoads, C. P.: J. Exper. Med. 61 · 173, 1935.
Wills, L , Clutterbuck, P. W., and Evans, B. D. F.: Biochem. J. 31: 2136, 1937.
Wintrobe, M. M.: Am. J. M. Sc 197: 286, 1939. Am. J. Physiol. 126 · 375, 1939.

Pyridoxine

Antopol, W., and Unna, K . Proc Soc Exper. Biol. & Med. 42: 126, 1939.
Antopol, W., and Schotland, C E · J A M A. 114 · 1058, 1940.
Chick, H., Macrae, T. F., Martin, A J P., and Martin, C. J.: Biochem. J. 32: 2207, 1938
Dimick, M. K., and Schreffler, C. B J. Nutrition 17 . 23, 1939.
Flexner, J., and Chassin, M R J. Clin. Invest 20. 313, 1941.
György, P. J Nutrition 16 69, 1938.
Harris, S. A., and Folkers, S K. Science 89 317, 1939.
Jolliffe, N.: Minn Med 23 542, 1940.
Kark, R., Lozner, E L , and Meiklejohn, A P Proc. Soc. Exper. Biol. & Med. 43 · 97, 1940
Kuhn, R., Westphal, K , Wendt, G , and Westphal, O.: Naturwissenschaften 27: 469, 1939.
Machella, T. E . Am. J Med Sc 203 114, 1942.
Morgan, A F Science 93 261, 1941.
Schneider, H , Steenbock, H., and Platz, B R J. Biol Chem. 132, 539, 1930.
Smith, S. G , and Martin, D W Proc Soc Exper Med & Biol 43 660, 1940.
Spies, T. D., Bean, W. B , and Ashe, W F J A M A 112 2414, 1939
Spies, T , Hightower, D P , and Hubbard, L H J A M A. 115 292, 1940.
Spies, T., Ladisch, R K., and Bean, W B J. A M A 115. 839, 1940.
Street, H R , Cowgill, G R , and Zimmerman, H M J Nutrition 21 · 275, 1941.
Thomas, R M., Mylon, E , and Winternitz, M C . Yale J Biol Med. 12. 345, 1940.
Vilter, S P., Spies, T D , and Mathews, A P J Biol Chem 125 · 85, 1938
Wintrobe, M M , Follis, R H., Jr , and others Bull. Johns Hopkins Hosp. 72: 1, 1943.

Pantothenic Acid

Ashburn, L. L · U S Pub Health Rep 55 · 1337, 1940
Brandaleone, H., Main, E , and Steele, M.. Proc Soc. Exper. Biol. & Med. 53: 47, 1943.
Daft, F. S., and Sebrell, W. H.. U. S. Pub Health Rep. 54 2247, 1939.
Daft, F. S., Sebrell, W. H , Babcock, S H , Jr , and Jukes, T. H.: U. S. Pub. Health Rep 55 1333, 1940.
Figge, F. H. J., and Soloman, K . J Lab & Clin. Med. 27: 1495, 1942
Gross, P., Harvalik, Z , and Runne, E · J. Invest. Dermatol 4. 385, 1941.
György, P., and Goldblatt, H.: J Exper Med 70. 185, 1939
György, P., Goldblatt, H , Miller, F. R., and Fulton, R. P.: J. Exper. Med. 66: 579, 1937.
Martin, G. J., and Ansbacher, S.: Proc. Soc. Exper. Biol. & Med. 48: 118, 1941.

MORGAN, A. F., AND SIMMS, H. D.: J. Nutrition 15·27, 1938.
PHILLIPS, P. H., AND ENGEL, R. W.: J. Nutrition 18.227, 1939.
RUSSELL, R. A., AND NASSET, E. S.: J. Nutrition 22: 287, 1941.
SPIES, T. D.: J. A. M. A. 115: 523, 1940.
WINTROBE, M. M., MILLER, M. H., AND OTHERS: J. Nutrition 24: 345, 1942.
WOOLEY, D. W.: J. Biol. Chem. 136: 113, 1940; Science 92: 384, 1940; Proc. Soc. Exper. Biol. & Med. 46: 565, 1941.

Biotin

CALDWELL, F. E, AND GYÖRGY, P.: Proc. Soc. Exper. Biol. & Med. 53·116, 1943.
LAURENCE, W. L.: Science 99, 392, 1944.
MEYER, K.. Science 99: 391, 1944.
OPPEL, T. W.: Am. J. Med Sc. 204: 856, 1942.
SULLIVAN, M., AND NICHOLLS, J.: Arch. Derm. & Syph. 45: 295, 1942.
SYDENSTRICKER, V. P., ET AL.: J. A. M. A. 118: 1199, 1942.
WILLIAMS, R. H.: New England J. Med. 228: 247, 1943.

Choline

BEST, C. H.: Science 94·523, 1941.
BEST, C. H., HERSHEY, J. M., AND HUNSTMAN, M. E.: Am. J. Physiol. 101: 7, 1932.
BLOOMBERG, E., AND McCOLLUM, E. V. Science 93: 598, 1941.
CHAIKOFF, I L., AND CONNOR, C. L: Proc. Soc. Exper. Biol. & Med. 43: 638, 1940.
ELVEHJEM, A. C.: *Handbook of Nutrition Chicago, Am. Med. Ass, 1943.*
FIELDS, H., AND WISE, E. C.: J. Clin. Invest. 18: 474, 1939.
GRAHAM, R. L.: Bull. Johns Hopkins Hosp. 74: 16, 1944.
GRIFFITH, W. H.: J. Biol. Chem. 131: 567, 1939.
GYÖRGY, P., AND ECKHARDT, R. E.: Biochem. J. 34: 1143, 1940.
JUKES, T. H.: J. Nutrition 20: 445, 1940.
RICH, A. R., AND HAMILTON, J. D.: Bull. Johns Hopkins Hosp. 66: 185, 1940

Cancer

ABELS, J. C., GORHAM, A. T., PACK, G. T., AND RHOADS, C. P.: J. Clin. Invest. 20: 749, 1941.
DE RAADT, O. L. E.: Ztschr. f. Krebsforsch. 30: 449, 1929.
DU VIGNEAUD, V., SPANGLER, J M, BURK, D., KENSLER, C. J., SUGIURA, K., AND RHOADS, C P. Science 95: 174, 1942.
JORSTAD, L. H.. J. Exper. Med. 42: 221, 1925.
KINOSITA, R.: Yale J. Biol. & Med. 12: 287, 1940.
KUH, C.: Yale J. Biol. & Med. 5: 123, 1932
RHOADS, C. P., ET AL (See Bull N. Y. Acad. Med 18: 53, 1942
SURE, B, BUCHANAN, K. S., AND THATCHER, H. S.: Am. J. Cancer 27: 94, 1936.

Cirrhosis

DAFT, F. S., SEBRELL, W. H, AND LILLIE, R. D.: Proc. Soc. Exper. Biol. & Med. 48: 228, 1941.
FLEMING, R. G., AND SNELL, A M.. Am. J. Dig. Dis. 9: 115, 1942.
FOUTS, P. J.: J. Nutrition 25: 217, 1943.
GYÖRGY, P., AND GOLDBLATT, H.: J. Exper. Med. 75: 355, 1942.
KENSLER, C. J, SUGIURA, K, YOUNG, N F, HALTER, C. R, AND RHOADS, C. P.: Science 93: 308, 1941.

ATEK, A. J., JR.: Bull. N. Y. Acad. Med. 19:498, 1943.
ICH, A. R., AND HAMILTON, J D.: Bull. Johns Hopkins Hosp. 66: 185, 1940.
ON GLAHN, W. C., AND FLINN, F. B : Am. J. Path. 15. 771, 1939.

Estrogen, Etc.

ECKS, H., WAINWRIGHT, W. W., AND MORGAN, A. F.: Am. J. Orthodont. & Surg. Orthodont. 29: 183, 1943.
ISKIND, G. R., AND MARK, J : Bull. Johns Hopkins Hosp 45:212, 1939.
ISKIND, M. S., BISKIND, G. R., AND BISKIND, L. H.: S. G. & O. 78: 49, 1944.
ISKIND, M. S., AND BISKIND, G. R.: Science 94: 462, 1941.

Cytopenia

ANGSTON, W. C , DARBY, W. J., SHUKERS, C. F., AND DAY, P. L.: J. Exper. Med. 68: 923, 1938.
OMLINSON (See Topping and Fraser).
OPPING, N. H., AND FRASER, H. F : U. S. Pub. Health Rep. 54: 416, 1939.

Congenital Malformations

ARKANY, J., AND NELSON, R. C : Science 92. 383, 1940.
ARKANY, J., AND SCHRAFFENBERGER, E : Proc. Soc. Exper. Biol. & Med. 54: 92, 1943.

Panmyelophthysis

AY, P. L , LANGSTON, W P., AND SHUKERS, C. F.: J. Nutrition 9: 637, 1935.
YÖRGY, P , GOLDBLATT, H , MILLER, F. R , AND FULTON, R. P.: J. Exper. Med. 66. 579, 1937.
ILLER, D. K., AND RHOADS, C. P · J Exper. Med. 61: 173, 1935.

Macrocytic Anemia

ASTLE, W B. et al. Science 100: 82, 1944
ILLS, L , CLUTTERBUCK, P W , AND EVANS, B. D. F.: Biochem. J. 31. 2136, 1937.
INTROBE, M. M.: Am. J. Med Sc. 197 286, 1939· Am. J. Physiol 126: 375, 1939.

PABA and the Sulfonamides

EE R. J. HENRY's review, Bact Rev. 7. 175, 1943.

CHAPTER XXI

Vitamin C Deficiency

BERCROMBIE, W. F Am. J Path. 11. 469, 1935
BT, A. F., FARMER, C J , AND EPSTEIN, I M : J. Pediat. 8: 1, 1936.
DANT, M.. Rev. belge sc med 10: 126, 1938
SCHOFF, L., AND KOCH, W., SKORBUT. Eine Pathologischanatomische Studie, Jena, Fischer, 1919
USPITZ, H.: System Der Hautkrankheiten, Vienna, Braumuller, 1881.
AITSELL, G. A.: J. Exper Med. 23: 739, 1916.
ARLOW, T : Med. Chir. Trans. 66· 159, 1883. Reprinted in Arch. Dis. Childhood 10, 1935
ARTON, W., AND FREEMAN, W.: New England J. Med. 210: 529, 1934.

MORGAN, A. F., AND SIMMS, H. D.: J. Nutrition 15: 27, 1938.
PHILLIPS, P. H., AND ENGEL, R. W.: J. Nutrition 18: 227, 1939.
RUSSELL, R. A., AND NASSET, E. S.: J. Nutrition 22: 287, 1941.
SPIES, T. D.: J. A. M. A. 115: 523, 1940.
WINTROBE, M. M., MILLER, M. H., AND OTHERS: J. Nutrition 24: 345, 1942.
WOOLEY, D. W.: J. Biol. Chem. 136: 113, 1940; Science 92: 384, 1940; Proc. Soc. Exp. Biol. & Med. 46: 565, 1941.

Biotin

CALDWELL, F. E., AND GYÖRGY, P.: Proc. Soc. Exper. Biol. & Med. 53: 116, 1943.
LAURENCE, W. L.: Science 99, 392, 1944.
MEYER, K.: Science 99: 391, 1944.
OPPEL, T. W.: Am. J. Med. Sc. 204: 856, 1942.
SULLIVAN, M., AND NICHOLLS, J.: Arch. Derm. & Syph. 45: 295, 1942.
SYDENSTRICKER, V. P., ET AL.: J. A. M. A. 118: 1199, 1942.
WILLIAMS, R. H.: New England J. Med. 228: 247, 1943.

Choline

BEST, C. H.: Science 94: 523, 1941.
BEST, C. H., HERSHEY, J. M., AND HUNSTMAN, M. E.: Am. J. Physiol. 101: 7, 1932.
BLOOMBERG, E., AND McCOLLUM, E. V.: Science 93: 598, 1941.
CHAIKOFF, I. L., AND CONNOR, C. L.: Proc. Soc. Exper. Biol. & Med. 43: 638, 1940.
ELVEHJEM, A. C.: Handbook of Nutrition. Chicago, Am. Med. Ass., 1943.
FIELDS, H., AND WISE, E. C.: J. Clin. Invest. 18: 474, 1939.
GRAHAM, R. L.: Bull. Johns Hopkins Hosp. 74: 16, 1944.
GRIFFITH, W. H.: J. Biol. Chem. 131: 567, 1939.
GYÖRGY, P., AND ECKHARDT, R. E.: Biochem. J. 34: 1143, 1940.
JUKES, T. H.: J. Nutrition 20: 445, 1940.
RICH, A. R., AND HAMILTON, J. D.: Bull. Johns Hopkins Hosp. 66: 185, 1940.

Cancer

ABELS, J. C., GORHAM, A. T., PACK, G. T., AND RHOADS, C. P.: J. Clin. Invest. 2 749, 1941.
DE RAADT, O. L. E.. Ztschr. f. Krebsforsch. 30: 449, 1929.
DU VIGNEAUD, V., SPANGLER, J. M., BURK, D., KENSLER, C. J., SUGIURA, K., AND RHOADS, C. P. Science 95: 174, 1942.
JORSTAD, L. H.: J. Exper. Med. 42: 221, 1925.
KINOSITA, R.: Yale J. Biol. & Med. 12: 287, 1940.
KUH, C.: Yale J. Biol & Med. 5: 123, 1932.
RHOADS, C. P., ET AL. (See Bull. N. Y. Acad. Med. 18: 53, 1942.
SURE, B., BUCHANAN, K S., AND THATCHER, H. S.: Am. J. Cancer 27: 94, 1936.

Cirrhosis

DAFT, F. S., SEBRELL, W. H., AND LILLIE, R. D.: Proc. Soc. Exper. Biol. & Med. 48: 228, 1941.
FLEMING, R. G., AND SNELL, A. M : Am J. Dig. Dis. 9: 115, 1942
FOUTS, P. J.: J. Nutrition 25. 217, 1943.
GYÖRGY, P., AND GOLDBLATT, H. J. Exper. Med. 75: 355, 1942.
KENSLER, C. J., SUGIURA, K., YOUNG, N. F., HALTER, C. R., AND RHOADS, C. P.: Science 93: 308, 1941.

PATEK, A. J., JR.: Bull. N. Y. Acad. Med. 19: 498, 1943
RICH, A. R., AND HAMILTON, J. D.: Bull. Johns Hopkins Hosp. 66: 185, 1940.
VON GLAHN, W. C., AND FLINN, F. B.: Am. J. Path. 15: 771, 1939.

Estrogen, Etc.

BECKS, H., WAINWRIGHT, W. W., AND MORGAN, A. F.: Am. J. Orthodont. & Surg. Orthodont. 29: 183, 1943.
BISKIND, G. R., AND MARK, J.: Bull. Johns Hopkins Hosp. 45: 212, 1939.
BISKIND, M. S., BISKIND, G. R., AND BISKIND, L. H.: S. G. & O. 78: 49, 1944.
BISKIND, M. S., AND BISKIND, G. R.: Science 94: 462, 1941.

Cytopenia

LANGSTON, W. C., DARBY, W. J., SHUKERS, C. F., AND DAY, P. L.: J. Exper. Med. 68: 923, 1938.
TOMLINSON (See Topping and Fraser).
TOPPING, N. H., AND FRASER, H. F.: U. S. Pub. Health Rep. 54: 416, 1939.

Congenital Malformations

WARKANY, J., AND NELSON, R. C.: Science 92: 383, 1940.
WARKANY, J., AND SCHRAFFENBERGER, E.: Proc. Soc. Exper. Biol. & Med. 54: 92, 1943.

Panmyelophthysis

DAY, P. L., LANGSTON, W. P., AND SHUKERS, C. F.: J. Nutrition 9: 637, 1935.
GYÖRGY, P., GOLDBLATT, H., MILLER, F. R., AND FULTON, R. P.: J. Exper. Med. 66: 579, 1937
MILLER, D. K., AND RHOADS, C. P.: J. Exper. Med. 61: 173, 1935.

Macrocytic Anemia

CASTLE, W. B. et al. Science 100. 82, 1944.
WILLS, L., CLUTTERBUCK, P. W., AND EVANS, B. D. F.: Biochem. J. 31: 2136, 1937.
WINTROBE, M. M.: Am. J. Med. Sc. 197 286, 1939 Am. J. Physiol. 126: 375, 1939.

PABA and the Sulfonamides

SEE R. J. HENRY's review, Bact. Rev. 7 175, 1943

CHAPTER XXI

Vitamin C Deficiency

ABERCROMBIE, W. F.: Am. J. Path. 11. 469, 1935
ABT, A. F., FARMER, C. J., AND EPSTEIN, I. M. J. Pediat. 8: 1, 1936.
ADANT, M. Rev. belge sc. med. 10: 126, 1938.
ASCHOFF, L., AND KOCH, W., SKORBUT Eine Pathologischanatomische Studie, Jena, Fischer, 1919.
AUSPITZ, H.: System Der Hautkrankheiten, Vienna, Braumuller, 1881.
BAITSELL, G. A.: J. Exper. Med. 23: 739, 1916.
BARLOW, T.: Med. Chir. Trans. 66: 159, 1883. Reprinted in Arch. Dis. Childhood 10, 1935.
BARTON, W., AND FREEMAN, W.: New England J. Med. 210: 529, 1934.

Morgan, A. F., and Simms, H. D.: J. Nutrition 15: 27, 1938.
Phillips, P. H., and Engel, R. W.: J. Nutrition 18: 227, 1939.
Russell, R. A., and Nasset, E. S.: J. Nutrition 22: 287, 1941.
Spies, T. D.: J. A. M. A. 115: 523, 1940.
Wintrobe, M. M., Miller, M. H., and others: J. Nutrition 24: 345, 1942.
Wooley, D. W.: J. Biol. Chem. 136: 113, 1940; Science 92: 384, 1940; Proc. Soc. Exper. Biol. & Med. 46: 565, 1941.

Biotin

Caldwell, F. E., and György, P.: Proc. Soc. Exper. Biol. & Med. 53: 116, 1943.
Laurence, W. L.: Science 99, 392, 1944.
Meyer, K.: Science 99: 391, 1944.
Oppel, T. W.: Am. J. Med. Sc. 204: 856, 1942.
Sullivan, M., and Nicholls, J.: Arch. Derm. & Syph. 45: 295, 1942.
Sydenstricker, V. P., et al.: J. A. M. A. 118: 1199, 1942.
Williams, R. H.: New England J. Med. 228: 247, 1943.

Choline

Best, C. H.: Science 94: 523, 1941.
Best, C. H., Hershey, J. M., and Hunstman, M. E.: Am. J. Physiol. 101: 7, 1932
Bloomberg, E., and McCollum, E. V.: Science 93: 598, 1941.
Chaikoff, I. L., and Connor, C. L.: Proc. Soc. Exper. Biol. & Med. 43: 638, 1940
Elvehjem, A. C.: Handbook of Nutrition. Chicago, Am. Med. Ass , 1943.
Fields, H., and Wise, E C.: J. Clin. Invest. 18: 474, 1939.
Graham, R. L.: Bull. Johns Hopkins Hosp. 74: 16, 1944.
Griffith, W. H.: J. Biol. Chem. 131: 567, 1939.
György, P., and Eckhardt, R. E.: Biochem. J. 34: 1143, 1940.
Jukes, T. H.: J. Nutrition 20: 445, 1940.
Rich, A. R., and Hamilton, J. D.: Bull. Johns Hopkins Hosp. 66: 185, 1940

Cancer

Abels, J. C., Gorham, A. T., Pack, G. T., and Rhoads, C. P.: J. Clin. Invest. 20. 749, 1941.
de Raadt, O. L. E.: Ztschr. f. Krebsforsch. 30: 449, 1929.
Du Vigneaud, V., Spangler, J. M., Burk, D., Kensler, C. J., Sugiura, K., and Rhoads, C P.: Science 95: 174, 1942.
Jorstad, L. H.: J. Exper Med. 42: 221, 1925.
Kinosita, R.: Yale J. Biol. & Med. 12: 287, 1940
Kuh, C.: Yale J. Biol. & Med. 5: 123, 1932.
Rhoads, C. P., et al. (See Bull. N. Y. Acad. Med. 18: 53, 1942.
Sure, B., Buchanan, K. S., and Thatcher, H. S.: Am. J. Cancer 27: 94, 1936.

Cirrhosis

Daft, F. S., Sebrell, W. H., and Lillie, R. D.: Proc. Soc. Exper. Biol. & Med. 48: 228, 1941.
Fleming, R. G., and Snell, A M. Am. J. Dig. Dis. 9: 115, 1942.
Fouts, P. J.: J Nutrition 25: 217, 1943.
György, P., and Goldblatt, H : J. Exper. Med. 75: 355, 1942.
Kensler, C J., Sugiura, K., Young, N. F., Halter, C. R , and Rhoads, C P.: Science 93: 308, 1941.

Patek, A. J., Jr : Bull. N. Y. Acad. Med. 19 493, 1943.
Rich, A. R., and Hamilton, J. D.: Bull. Johns Hopkins Hosp 66. 185, 1940.
Von Glahn, W. C., and Flinn, F. B : Am. J. Path. 15: 771, 1939.

Estrogen, Etc.

Becks, H , Wainwright, W. W., and Morgan, A F.: Am. J. Orthodont. & Surg. Orthodont. 29: 183, 1943.
Biskind, G. R., and Mark, J.: Bull Johns Hopkins Hosp. 45: 212, 1939.
Biskind, M. S , Biskind, G. R., and Biskind, L. H.: S G. & O. 78: 49, 1944.
Biskind, M. S., and Biskind, G. R : Science 94: 462, 1941.

Cytopenia

Langston, W. C., Darby, W. J., Shukers, C. F., and Day, P. L.: J. Exper. Med. 68: 923, 1938.
Tomlinson (See Topping and Fraser).
Topping, N. H., and Fraser, H. F.: U. S. Pub. Health Rep. 54: 416, 1939.

Congenital Malformations

Warkany, J., and Nelson, R. C.· Science 92. 383, 1940.
Warkany, J., and Schraffenberger, E.: Proc Soc Exper. Biol. & Med. 54: 92, 1943.

Panmyelophthysis

Day, P. L., Langston, W P., and Shukers, C F : J. Nutrition 9: 637, 1935.
György, P., Goldblatt, H., Miller, F R., and Fulton, R P.: J. Exper. Med. 66: 579, 1937.
Miller, D. K., and Rhoads, C. P · J. Exper. Med. 61: 173, 1935.

Macrocytic Anemia

Castle, W. B et al· Science 100. 82, 1944
Wills, L , Clutterbuck, P. W , and Evans, B. D. F.: Biochem. J. 31. 2136, 1937.
Wintrobe, M. M · Am. J. Med. Sc 197· 286, 1939: Am. J. Physiol. 126: 375, 1939.

PABA and the Sulfonamides

See R. J. Henry's review, Bact. Rev. 7 175, 1943.

CHAPTER XXI

Vitamin C Deficiency

Abercrombie, W. F · Am. J. Path. 11. 469, 1935.
Abt, A. F., Farmer, C. J., and Epstein, I M.: J. Pediat. 8: 1, 1936.
Adant, M.. Rev. belge sc med. 10: 126, 1938.
Aschoff, L., and Koch, W , Skorbut. Eine Pathologischanatomische Studie, Jena, Fischer, 1919.
Auspitz, H.: System Der Hautkrankheiten, Vienna, Braumuller, 1881.
Baitsell, G. A.: J. Exper. Med. 23: 739, 1916
Barlow, T.: Med Chir. Trans. 66: 159, 1883. Reprinted in Arch. Dis. Childhood 10, 1935.
Barton, W., and Freeman, W.: New England J. Med. 210: 529, 1934.

BAUCKE, J.: *Pflugers Arch. f. d. ges. Physiol.* 241: 392, 1928.
BESSEY, O. A., MENTEN, M. L., AND KING, C. G.: Proc. Soc. Exper. Biol. & Med. 31: 455, 1934.
BEZSSONOFF, M. N.: Bull. soc. chim. biol. 11: 294, 1929 and 13: 950, 1931.
BOURNE, G.: Brit. M. J. 1: 560, 1938
BOYLE, P. E.: Am. J. Path 14: 843, 1938.
BOYLE, P. E., BESSEY, O. A., AND HOWE, P. R.: Arch. Path. 30: 90, 1940.
BRENNEMANN, J.: J. Michigan M. Soc 1923.
BUTLER, A. M., AND CUSHMAN, M.: J. Clin. Invest 19: 459, 1940
CHAMBERLIN, D. T., AND PERKIN, H. J.: Am. J. Digest. Dis. 5: 493, 1938.
CHAPMAN, O. D, AND HARRIS, A. E.: N. Y. State Ass. Pub. Health Labs., Albany, Nov. 1, 1940.
CHICK, H, AND HUME, E. M: J. Biol. Chem. 39: 203, 1919.
CHU, F., AND CHOW, B. F.: Proc Soc. Exper. Biol. & Med. 38: 679, 1939.
COHEN, B., AND MENDEL, L. B.: J. Biol. Chem. 35: 425, 1918
COLEMAN, W.: Am J. Med. Sc. 77, 1912. Also J. A. M. A. 64: 329, 1917.
CRANDON, J H, AND LUND, C. C.: *New Eng. J. Med.* 222: 748, 1940.
CROFT, J. D, AND SNORF, L. D.: J. Am. Med. Sc. 198: 403, 1939.
DALLDORF, G.: J. Exper Med. 53. 289, 1931.
DALLDORF, G.: Am J. Dis. Child. 46: 794, 1933
DALLDORF, G., AND RUSSELL, H: J. A. M. A. 104: 1701, 1935.
DALLDORF, G., AND ZALL, C: J. Exper. Med. 53: 289, 1931.
DANN, M., AND COWGILL, G. R.: J. Nutrition 9: 507, 1935
DARLING, S. T.: J. A. M. A 63: 1290, 1914.
DOI, J.: J. Orient. Med. 28: 209, 1938.
ECKER, E. E., AND PILLEMER, L.: J A. M. A. 112: 1449, 1939.
EDDY, W. H.: Am. J. Pub Health 19: *1309, 1930*
EDDY, W. H., AND KOHMAN, E. F.: J. Indust. Engin Chem. 16: 52, 53, 1924.
ELLIOTT, R. H E.: J A. M. A. 110: 1177, 1938.
ELMBY, A., AND WARBURG, E.: Lancet 2: 1363, 1937.
ERDHEIM, J Wien Klin Wchnschr 31: 1233, 1918.
EULER, R., AND OTTO, H.: Med. Klin 34: 709, 1938
FISH, E. W., AND HARRIS, L. J.: Brit Dent. J. 58: 3, 1935.
FORBUS, W D Arch. Path 2 318 and 468, 1926.
FRÖLICH, T.: Ztschr f. Hyg u Infectionskrankh. 72: 155, 1912.
GIROUD, A.: Compt rend. Soc. de Biol. 120: 701, 1935.
GIROUD, A: Protoplasma-Monographien 16. Berlin, Gebruder Borntraeger, 1938.
GIROUD, ET AL.: Compt. rend. Soc de Biol. 120: 414, 1935 (vitamin values in non-susceptible species).
GLASUNOW, M Virchows Arch. f. path Anat. 299: 120, 1937.
GLICK, D, AND BISKIND, G R J Biol. Chem. 110: 1 and 583, 1935.
GOETTSCH, E.: Am. J Dis Child. 49: 1411, 1935
GÖTHLIN, G F: J Lab & Clin Med. 18: 484, 1933. Also Lancet, 2: 703, 1937.
GYÖRGY, P., in Avitaminosen u verwandte Krankheitzustande, Berlin, Springer, 1927.
HAM, A. W., AND ELLIOTT, H C: Am. J. Path 14: 323, 1938.
HARDEN,—, AND ZILVA,— (quoted from The Vitamins, H C. Sherman and S. L. Smith, New York, The Chemical Catalog Company, 1931).
HARMAN, M T, AND KRAMER, M M.: J Nutrition 15. 277, 1938.
HARRIS, L. J., ABBASY, M A., AND YUDKIN, J.: Lancet 1 1488, 1936.

HAWORTH, W. N., HIRST, E. L., AND ZILVA, S. S.: J. Chem. Soc. London 2: 1155, 1934.
HECHT, A. F.: Jahrb. f. Kinderh. 65: 113, 1907.
HERTZLER, A. E.: Anat. Rec. 9: 84, 1915.
HESS, A. F.: Scurvy, Past and Present, Philadelphia, Lippincott, 1920. Also J. A. M. A. 98: 1429, 1932 (etiology).
HILL, A. L.: Arch. Pediat. 45: 251, 1932.
HJÄRRE, C. A., AND LILLEENGEN: Virchows Arch. f. path. Anat., 297: 565, 1936.
HOFFMAN: Jahrb. F. Kinderh. 98, 1922 (quoted by P. György).
HÖJER, A.: Studies in Scurvy, Acta paediat. 8 (suppl. 3), 1924.
HOLST, A., AND FRÖLICH, T.: J. Hyg. 7: 634, 1907.
INGALLS, T. H., AND WARREN, H. A.: New England J. Med. 217: 443, 1937.
INGIER, A.: J. Exper. Med. 21: 525, 1915.
ISRAELS, M. C. G.: Lancet 244: 17, 1943.
JACKSON, D., AND PARK, E. A.: J. Pediat. 7: 741, 1935.
JACKSON, L., AND MOORE, J. S.: J. Infect. Dis. 19: 478, 1916.
KAJDI, L., LIGHT, L., AND KAJDI, C.: J. Pediat. 15: 197, 1939.
KING, C. G.: Physiol. Rev. 16: 238, 1936.
KOLLATH, W.: Klin. Wchnschr. 10: 1841, 1931.
LAZARUS, S.: Brit. M. J. 2: 1011, 1937.
LEPKOVSKY, S., AND NELSON, M. T.: J. Biol. Chem. 59: 91, 1924.
LEVINE, S. Z., GORDON, H. H., AND MARPLES, E.: J. Clin. Invest. 20: 209, 1941.
MAHÉ, J.: Scorbut in Dictionnaire encyclopédique des sciences médicales (Dechambre), series 3, 8: 35, 1880.
MAZOUE, H., AND RANDOIN, L.: Ann. de physiol. 13: 1057, 1937.
McCOLLUM, E. V., AND PITZ, W.: J. Biol. Chem. 31: 229, 1917.
MEDES, G.: Proc. Soc. Exper. Biol. & Med. 23: 294, 1926.
MEYER (quoted by P. György)
MEYER, A. W., AND McCORMICK, L.: Studies in Scurvy, Stanford Univ. Press, 1928.
MEYER, L. F., AND NASSAU, E.: Jahrb. f. Kinderh. 94: 341, 1921.
MOORE, J. J., AND JACKSON, L.: J. A. M. A. 67: 1931, 1916.
MOURIQUAND, G., AND DAUVERGNE, M.: J. méd. franc. [illegible]: 411, 1938.
MOURIQUAND, G., EDEL, V., DAUVERGNE, M., AND LAVAUD, J.: Compt. rend. Soc. de Biol. 129: 673, 1938.
MOURIQUAND, POUZET, SCHOEN, AND BELLY: Lyon méd. 152: 180, 1933.
MÜLLER, E.: Centralbl. f. allg. Path. u. path. Anat. 58: 305, 1933.
MUSELIN, R. R., TULLY, R. H., LONGNECKER, H. E., AND KING, C. G.: Science 88: 552, 1938.
NIELSEN, H. E.: Bibliot. f. laeger 130: 20, 1938.
PARK, E. A., GUILD, H. G., JACKSON, D., AND BOND, M.: Arch. Dis. Childhood 10: 265, 1935.
P[illegible]
P[illegible]
P[illegible]
RATNOFF: Ztschr. f. Kinderh. 34, 1923 (quoted by P. György).
REICHSTEIN, T., GRÜSSNER, A., AND OPPENAUER, R.: Helvet. chim. acta, Basel 16: 1019, 1933.
[illegible] Ibid. 15: 25, 1939.

Pappenheimer, A. M.: J. Exper. Med. 36: 335, 1922 (histopathology). Ibid., J. Exper. Med. 64: 965, 1936 ("renal rickets"). Ibid., J. Exper. Med. 52: 805, 1930 (parathyroidectomy).
Park, E. A.: The Vitamins, Chicago, Am. Med. Ass., 1939. Ibid., Bull. New York Acad. Med. 15: 495, 1939 (histopathology).
Pommer, G.: Untersuchungen über Osteomalacie und Rachitis, Leipzig, 1885.
Reed, C. I.: J. A. M. A. 102: 1745, 1934.
Schultz, O.: Klin. Wchnschr. 12: 114, 1933.
Sebrell, W. H.: Handbook of Nutrition. Chicago, Am. Med. Ass., 1943.
Shelling, D. H., and Asher, D. E.: Johns Hopkins Hosp. Bull. 50: 318, 1932.
Shelling, D. H., and Hopper, K. B.: Bull. Johns Hopkins Hosp. 58, 137, 1926.
Sherman, H. C., and Pappenheimer, A. M.: Proc. Soc. Exper. Biol. & Med. 18: 193, 1920.
Shohl, A. T.: The Vitamins, Chicago, Am. Med. Ass., 1939. Ibid., New England J. Med. 220: 515, 1935 (cure with citrate).
Stockard, C.: Bull. New York Acad. Med. (Pediat. Sect) Jan. 12, 1928.
Tanret, C.: Compt. rend. 108: 98, 1889.
Thompson, J.: J. Nutrition 5: 359, 1932.
Toverud, G : J. Biol. Chem. 58: 583, 1923.
Toverud and Guttorm: Thesis, Oslo, 1926.
Trousseau, A.: Clinical Medicine. Philadelphia, 1882.
Vanderveer, H. L.: Arch. Path. 12: 941, 1931.
Vollmer, H.. J. Pediat. 14: 491, 1939.
Wahlgreen, F., Herlitz, C. W., and Jundell, I.: Acta. paediat 8: 443, 1929.
Wells, H. G., and Holley, S. W.: Arch. Path. 34 435, 1942.
Wesson, L. G., and Boyle, P. E. Arch. Path. 35: 237, 1943.
Wolbach, S. B., see Shohl, A. T., and Wolbach, S. B.: J. Nutrition 11: 275, 1936.
Wolfe, J. J.: Am. J. Dis. Child. 49: 905, 1935.

CHAPTER XXIII

Vitamin E Deficiency

Adamstone, F. B.: J. Morphol. & Physiol. 52: 47, 1931 (egg embryo).
Adamstone, F. B.: Arch. Path. 31. 603, 613, 622, 706, 711, 1941.
Adamstone, F. B., and Card, L. E.: J. Morphol. & Physiol. 56: 339, 1934 (fowl testis).
Anderson, H. D , Elvehjem, C. A., and Gonce, J. E , Jr.: Proc. Soc. Exper. Biol. & Med. 42: 750, 1939.
Barrie, M. M. O.: Biochem. J. 32: 2134, 1938.
Bicknell, F.. Lancet 238. 10, 1940.
Bicknell, F. Lancet 1: 10, 1940 Also The Vitamins in Medicine, Bicknell and Prescott, Heineman, 1942.
Bird, H. R., and Culton, T. G : Proc. Soc. Exper. Biol. & Med. 44: 543, 1940.
Butt, H. R.: Handbook of Nutrition. Chicago, Am. Med Ass., 1943.
Carruthers, C. Proc. Soc. Exper. Biol & Med. 40: 107, 1939.
Chor, H , and Dalkart, R. E . Arch. Path. 27 497, 1939.
Cromer, J. K.: M. Ann District of Columbia 7: 145, 1938.
Currie, D. W.: Brit. M. J 1 752, 1936. Ibid. 2: 1218, 1937.
Daft, F., Endicott, K M , Ashburn, L L., and Sebrell, W. H.: Proc. Soc. Exper. Biol. & Med. 53: 130, 1943.
Dam, H.. J. Nutrition 27: 193, 1944.

DAM, H., AND GLAVIND, J.: Die Naturwissenschaften 28: 207, 1940. Science 96: 235, 1942
DAM, H., GLAVIND, J., BERNTH, O., AND HAGENS, E.: Nature No. 3609, 1938.
DAVISON, C : Bull. N. Y. Acad. Med. 19, 386, 1943.
DI BIASI, W.: Virchows Arch f. path. Anat 275: 250, 1930
EVANS, H. M., AND BURR, G. O.: Memoirs Univ. Cal. 8: 1, 1927. J. Biol. Chem. 76: 273, 1928.
EVANS, H. M., AND EMERSON, G A : Proc. Soc. Exper. Biol. & Med. 41: 318, 1939 (tumor).
EVANS, H. M, AND EMERSON, G A · *Science 89: 438, 1939.*
GOETTSCH, M., AND PAPPENHEIMER, A M.· J. Exper. Med 54: 145, 1931. Also separate reports in Proc Soc Exper. Biol. & Med. Vol. 27, 1930.
HARRIS, P. L. (quoted by Hickman).
HICKMAN, K · Ann Rev Biochem Vol 12, 1943
HOUCHIN, O B, AND MATTILL, H A J Biol. Chem. 146: 309, 1942.
JUHASZ-SHÄFFER, A Virchows Arch f path. Anat. 281. 53, 1931. Ibid. 282: 662, 1931
JUNGHERR, E.· Science 84 559, 1936
JUNGHERR, E, AND PAPPENHEIMER, A M. Proc Soc. Exper Biol. & Med. 37: 520, 1937
KAUNITZ, H, AND PAPPENHEIMER, A M · Am J Physiol. 138: 328, 1943.
KIHN, B Avitaminosen und Verwandte Krankheitszustände. Berlin, Springer, 1927
KNOWLTON, G C., HINES, H M, AND BRINKHOUS, K M.: Proc. Soc. Exper. Biol. & Med 42 804, 1939
KUDRJASCHOV, B A. Endokrinologie 7 · 91, 1930.
MACKENZIE, C. G, MACKENZIE, J B, AND MCCOLLUM, E. V.. Proc. Soc. Exper. Biol & Med. 44 95, 1940.
MACKENZIE, C G, AND MCCOLLUM, E V · Science 89 371, 1939.
MADSEN, L L, MCCAY, C M, AND MAYNARD, L. A : Proc. Soc. Exper. Biol. & Med. 30 1434, 1933 J. Nutrition 11. 471, 1936
MALPAS, P : J Obst. Gyn Brit Emp 49 82, 1942.
MASON, K E · Am J. Anat 52· 153, 1933. Essays in Biology, Univ. Cal. Press, 1943.
MASON, K. E, AND BRYAN, W L Biochem J. 32· 1785, 1938.
MORGULIS, S, AND SPENCER, H C J Nutrition 11 573, 1936.
MORGULIS, S, WILDER, V M, AND EPPSTEIN, S. H.· J. Nutrition 16: 219, 1938.
PAPPENHEIMER, A. M : Am J Path 15· 179, 1935 (histopathology); 18. 169, 1942 (mouse), Physiol Rev 23 37, 1943 (nervous lesions).
PAPPENHEIMER, A M, AND GOETTSCH, M · J. Exper Med. 53: 11, 1931.
PAPPENHEIMER, A M, AND GOETTSCH, M.· Proc Soc Exper. Biol. & Med. 43: 313, 1940.
POULSSON, E. Münch med Wchnschr. 74· 674, 1927.
RINGSTED, A : Biochem J 29 788, 1935.
ROWNTREE, L G., LANSBURG, J, AND STEINBERG, A.. Proc. Soc. Exper. Biol. & Med. 36: 421, 1937
SEIFRIED, O., AND HEIDEGGER, E.. Arch. f. wiss und prakt Tierhlk. 70: 122, 1936.
SHIMOTORI, N., EMERSON, G. A, AND EVANS, H. M Science 90. 89, 1939.
SHUTE, E.: Am. J. Obst & Gynec. 33: 429, 1937. Also J. A. M. A. 110. 889, 1938 (vaginitis).
STÄHLER, F., AND PEHL, B.: Arch. Gynak. 171. 134, 1941.

STEINBERG, C. L.: New York State J. Med. 42: 773, 1942.
STONE, S.: J. A. M. A. 114: 2187, 1940.
THOMAS, B. H., CANNON, C. Y., McNUTT, S. H., AND UNDERBJERG, G.: J. Nutrition 15: 10, 1938.
VAN WAGENEN, G.: Anat. Rec. 29: 398, 1925.
VERZAR, F.: Proc. Staff Meet.; Mayo Clin. 4: 351, 1929. Ibid., Schweiz. med. Wchnschr. 69: 738, 1939. Ibid, Ztschr. f. Vitaminforsch. 9: 242, 1939 (creatinuria).
VOGT-MØLLER, P.: Lancet 2: 182, 1931. Klin. Wchnschr. 15: 1883, 1936.
WATSON, E. M., AND TEW, W. P.: Am. J. Obst. & Gynec. 31: 352, 1936
WECHSLER, I. S.: J. A. M. A. 114: 948, 1940.

CHAPTER XXIV

Vitamin K Deficiency

AGGELER, P. M., LUCIA, [redacted] P., AND GOLDMAN, L.: Proc. Soc. Exper. Biol. & Med. 43: 689, 1940.
ANDRUS, W. D., AND LORD, J. W.: J. A. M. A. 114: 1336, 1940.
ANDRUS, W. D., LORD, J. W., JR., AND KAUER, J. T.: Science 91: 48, 1940.
BRINKHOUS, K. M., SMITH, H. P., AND WARNER, E. D.: Am. J. M. Sc. 193: 475, 1937.
BUTT, H. R., SNELL, A. M., AND OSTERBERG, A. E.: Proc. Staff Meet., Mayo Clin. 13: 74, 1938.
DAM, H.: Biochem. Ztschr. 215: 475, 1929. Ibid. 220: 158, 1930.
DAM, H., AND GLAVIND, J.: Acta. med. Scandinav. 96: 108, 1938.
DAM, H., AND SCHØNHEYDER, F.: Biochem. J. 28: 1355, 1934 (lesions in chicks).
DAM, H., SCHØNHEYDER, F., AND LEWIS, L.: Biochem. J. 31: 22, 1937.
ELLIOTT, M. C., ISAACS, B., AND IVY, A. C.: Proc. Soc. Exper. Biol. & Med. 43: 240, 1940.
FLYNN, J. E., AND WARNER, E. D.: Proc. Soc. Exper. Biol & Med 43: 190, 1940.
GREAVES, J. D., AND SCHMIDT, C. L. A.. Proc. Soc. Exper. Biol. & Med. 37: 43, 1937.
KARK, R., AND LOZNER, E. L: Lancet 237: 1162, 1939.
MACKIE, T. T.: New York State J. Med 40: 987, 1940.
McFARLANE, W. D., GRAHAM, W. R., AND RICHARDSON, F.: Biochem. J. 25: 358, 1931
QUICK, A. J.: J. A. M. A. 109: 66, 1937.
QUICK, A. J.. Proc. Am. Soc Biol. Chem. 34: 78, 1940
QUICK, A. J., AND GROSSMAN, A M.: Am J. M. Sc. 199: 1, 1940.
SCARBOROUGH, H.: Lancet 1: 1080, 1940.
[illegible] Am. J. Digest. Dis 5: 590, 1938.
[illegible] 1939.
[illegible] 1939.
WARNER, E. D., BRINKHOUS, K. M., AND SMITH, H. P.: Proc. Soc. Exper. Biol. & Med. 37. 628, 1937.

CHAPTER XXV

Infectious Diseases

ANDERSON, O.: Acta paediat 14: 81, 1932.
ASCHOFF, L., AND KOCH, W.: Skorbut. Eine Pathologisch-anatomische Studie, Jena, Fischer, 1919.

Badger, L. F., and Masunaga, E.: U. S. Pub. Health Rep 55: 1027, 1940.
Beard, H. H.: J. Am. Dietet. Ass 10: 193, 1934.
Birkhaug, K. E.: Acta Tuberculosea Scand 13: 45, 1939.
Bloomfield, A. L., and Lew, W.: J. Nutrition 25: 427, 1943.
Borsalino, G.: Giornale di Clin. Med. 18: 213, 1937.
Boynton, L. C., and Bradford, W. L.: J. Nutrition 4: 323, 1931.
Clausen, S. W.: J. A. M. A. 101: 1385, 1933. Ibid., Am. J. Dis Child. 42: 698, 1931.
Clausen, S. W., and McCoord, A. B.: J. Pediat. 13: 635, 1938.
Daum, K., Boyd, K., and Paul, W. D: Proc. Soc. Exper. Biol. & Med. 40: 129, 1939.
Faulkner, L. M., and Taylor, F. H. L.: Ann. Int. Med. 10: 1867, 1937.
Findlay, G. M.: Brit. J. Exper. Path 6: 16, 1925.
Fleming, A: Proc. Roy Soc. Med. 93: 306, 1922.
Gardner, E. F., and Gardner, F. W.: Am. J Dis Child. 47: 1261, 1934.
Harde, E.: Compt. rend de l'Acad d sc. 199: 618, 1934.
Heaslip, W. G.: Australian J Exper. Biol & Med. Sc. 16: 287, 1938.
Heise, F. H., and Martin, G L Proc. Soc. Exper. Biol. & Med. 35: 337, 1936.
Heise, F. H., Martin, G. J., and Schwartz, S: Brit J. Tuberculosis 31: 23, 1937.
Hess, A. F., and Benjamin, L H J A M. A. 10 657, 1933
Höjer, A: Studies in Scurvy, Acta paediat., 8 (suppl 3), 1924.
Holmes, A. D., et al J Ind & Eng Chem 24: 1058, 1932.
Jungeblut, C. W J Exper Med. 66 459, 1937
Jungeblut, C. W, and Zwemer, R L Proc. Soc. Exper. Biol. & Med. 32: 1229, 1935.
Jusatz, H L.: Ztschr f Immunitatsforschung 88. 472, 1936
King, C. G, and Menten, M L J Nutrition 10 129, 1935. See also Sigal, A., and King, C. G J Pharm & Exper Ther 59. 468, 1937.
Kuczynski, M The Alimentary Factor in Disease, The Hague, G Naeff, 1937.
Kumagai, K., J A M A 109 601, 1937
Lamb, A. R: Am. J. Hyg 21 438, 1935
Lassen, H. C. A: J Hyg 30 300, 1930 Ibid, Ztschr. f. Immunitatsforschung u. Exp. Ther. 73 221, 1932
Mazoué, H Compt rend Soc de biol 126. 991, 1937
McCarrison, R. Studies in Deficiency Disease, Frowde, Hodder and Stoughton, 1921.
McClung, L. S., and Winters, J C J Infect Dis 51: 469, 1932.
Mellanby, E Brit Med J 1 984, 1929 Ibid, Nutrition and Disease, London, Oliver and Boyd, 1934
Pakter, J., and Schick, B: Am J Dis Child 55 1, 1938.
Perla, D.: Arch Path 25 539 & 691, 1938 (Vitamin C and infectious disease reviewed by Perla and Marmorston, J, Arch Path. 23. 543 & 683, 1937).
Pinkerton, H., and Bessey, O A Science 89: 368, 1939.
Prickett, P. S., Miller, N J, and McDonald, F. G.: J. Bact 33: 39, 1937
Rasmussen, A. F., Waisman, H. A., Elvehjem, C. A., and Clark, P. F.: J. Bact. 45: 85, 1943.
Rinehart, J. F., for references and summary see Rinehart, J. F., Greenberg, L. D., Baker, F., Mettier, S R, Bruckman, F. and Choy, F., Arch. Int. Med. 61: 537, 1938.
Robertson, E. L.: Medicine 13: 123, 1934.
Sabin, A.: J. Exper. Med 69. 507, 1939.
Schultz, M. P.: U. S. Pub. Health Rep. 54: 1205, 1939.

Schultz, M. P., and Rose, E. J.: U. S. Pub. Health Rep. 54: 527, 1939.
Sherman, H. C., and MacLeod, F. L.: J. Am. Chem. Soc. 47: 1658, 1925.
Shibley, G. S., and Spies, T. D.: J. A. M. A. 103: 2021, 1934.
Smith, T., Twelfth and Thirteenth Annual Report, Bureau of Animal Industry, 1895–96 (page 172).
Steinbach, M. M.: Am. Rev. Tuberculosis 26: 52, 1932.
Steinbach, M. M., and Klein, S. J.: Proc. Soc. Exper. Biol. & Med. 35: 151, 1936.
Sullivan, N. P., and Manville, I. A.: Am. J. Pub. Health 27: 1108, 1937.
Taylor, S.: Lancet 1: 973, 1937.
Torrance, C. C.: J. Biol. Chem. 121: 31, 1937. Ibid., J. Biol. Chem. 132: 575, 1940. Ibid., Proc. Soc. Exper. Biol. & Med. 41: 421, 1939.
Wachsmuth, W. and Heinreich, G.: Klin. Wchnschr. 17: 269, 1938.
Wolbach, S. B.: Growth and Development of the Child. Part III. Nutrition, New York, The Century Company, 1932.
Zinsser, H., Ruiz Castaneda, M., and Seastone, C. V., Jr.: J. Exper. Med. 53: 333, 1931.
Zook, J., and Sharpless, G. R.: Proc. Soc. Exper. Biol. & Med. 39: 233, 1938.

APPENDIX

REFERENCES USED IN ESTIMATING VITAMIN POTENCIES

The vitamin value of raw foods in Tables I and II following are given in approximate quantity of specific vitamins per 100 grams of raw natural foods (edible portion) based on reports in current literature. The numbers following the statement of quantity refer to the source articles given below.

(1) Misc. Publication #505, U. S. Dept. Agric.; E. M. Hewston and R. L. Marsh.
(2) Univ. Texas Publ. 4327, Oct. 1, 1942; V. H. Cheldelin and R. J. Williams.
(3) J. of Nutrition 23: 613, 1942; R. L. Lane, E. Johnson, R. R. Williams. J. of Nutrition 26: 417, 1943; V. Cheldelin and R. R. Williams.
(4) J. of Nutrition 24: 235, 1942; R. W. McVicar and G. H. Berryman.
(5) Food Research J. 7: 85, 1942; Hazel Munsell.
(6) J. of Nutrition 24. 441, 1943; R. W. Engel.
(7) J. of Nutrition 25: 463, 1943; R. A. Sullivan, E. Bloom, J. Jarmol.
(8) J. of Nutrition 25: 265, 1943; R. R. Sealock, A. H. Livermore.
(9) J. of Nutrition 26: 319, 1943; M. H. Haydak, L. S. Palmer, M. C. Tanquary, A. E. Vivino.
(10) J. of Nutrition 24: 167, 1942; L. J. Teply, F. M. Strong, C. A. Elvehjem.
(11) J. of Nutrition 24: 85, 1942; M. I. Bailey and A. W. Thomas.
(12) Tecn. Bull. #707, U. S Dept. Agric., Dec. 1939; L. Booher and E. R. Hartzler.
(13) Milbank Memorial Quarterly, 18: 311, 1940; Hazel Munsell.
(14) Food Research J. 5: 395, 1940, A. Z. Hodson.
(15) Food Research J. 1: 121, 1936; I. A. Manville, A. S McMinis, P. G. Chuinard.
(16) Food Research J. 5. 435, 1940, L. W. Todhunter.
(17) Food Research J. 4: 317, 1939; P. L. Harris and G. L. Poland.
(18) Food Research J. 2: 311, 1937; P. L. Harris and G. L. Poland.
(19) Food Research J. 1: 501, 1936; O A. Merriam and C. R. Fellers.
(20) Food Research J. 4: 593, 1939; K. Wheeler, D. K. Tressler, and C. G. King.
(21) Food Research J. 3: 403, 1938; F. Fenton, D. K. Tressler, S. C. Camp, C. G. King.
(22) Food Research J. 2: 175; 1937; D K Tressler, G. L Mack, R. R. Jenkins.
(23) Food Research J. 4· 309, 1939; G. L. Mack, W. T. Tapley, C. G. King.
(24) Food Research J. 5: 93, 1940, W I. Zimmerman, D. K. Tressler, L. A. Maynard.
(25) Food Res. 5: 253, 1940; E. Kelly, S Dietrich, T. Porter.
(26) Food Research J. 6· 57, 1941; W. I Zimmerman, D. K. Tressler, L. A. Maynard.
(27) Food Research J. 6: 85, 1941, E. Kelly, T. Porter.
(28) Food Research J. 7. 171, 1942; K. Farrel, C. R. Fellers.
(29) Food Research J. 1· 427, 1936, S. Gould, D. K. Tressler, C. G. King.
(30) Food Research J. 3: 311, 1938; M. Wellington, D. K. Tressler.
(31) Food Research J. 5· 247, 1940, R C. Burrell, H. O. Brown, V. R Ebright.
(32) Food Research J. 4· 31, 1939; C. S. Pederson, G. L. Mack, W. L. Athawes.
(33) Food Research J. 4· 371, 1939, W. C. Sherman, W. D. Salmon.
(34) Food Research J. 2: 41, 1937; C F. Dunker, C. R. Fellers, G. A. Fitzgerald.
(35) Food Research J. 2 331, 1937; J. A. Roberts.
(36) Food Research J. 5· 233, 1940; E. Metcalfe, P. Rehn, J. Winters.
(37) Food Research J. 6: 175, 1941; A Z. Hodson.
(38) Food Research J. 4 145, 1939, A. F. Morgan, L Kimmél, H. C. Davison
(39) Food Research J. 3. 109, 1938; G. A. Fitzgerald, C. R. Fellers.
(40) Food Research J. 7. 382, 1942, W. W. Floyd, G. S. Fraps.

(41) Food Research J. 4: 217, 1939; A. F. Morgan, H. L. Nobles, A. Wiens, G. L. Marsh, and A. J. Winkler.
(42) Food Research J. 2 81, 1937; J. E. Richardson, R. Davis, P. Sullivan.
(43) Food Research J. 1: 223, 1936; M. J. Mack, C. R. Fellers, W. A. Maclinn, D. A. Bean.
(44) Food Research J. 6: 581, 1941; E T. Murphy.
(45) Food Research J. 6: 373, 1941; D. K. Tressler, J. C. Moyer.
(46) Food Research J. 7: 218, 1942; E J. Brown, F. Fenton.
(47) Food Research J. 6· 396, 1941; M. M. Kirk, D. K. Tressler.
(48) Food Research J. 6. 217, 1941; E. J. Brown, H. Schuele, F. Fenton.
(49) Food Research J. 2 85, 1937; J E. Richardson, R. Davis, H. L. Mayfield.
(50) Food Research J. 5 241, 1940; G. H. Satterfield, M. Yarbrough.
(51) Food Research J. 1: 341, 1936, E. P. Daniel, M B. Rutherford.
(52) Food Research J. 1 348, 1936; P. L. Day, W. J. Darby.
(53) Food Research J. 5. 33, 1940, W W Floyd, G S. Fraps.
(54) Food Research J 7 141, 1942; M. E Puffer, W. F. Hinman, H. Charley, E. G. Halliday.
(55) Food Research J 1 231, 1936, G. L Mack, D. K Tressler, C. G. King.
(56) J. Nutrition 12 285, 1936; F Fenton, D. K Tressler, C. G. King.
(57) Food Research J 3 489, 1938, E N Todhunter, B. L. Sparling.
(58) Food Research J 4 475, 1939; C. R. Stimson, D. K Tressler, L. A. Maynard.
(59) Food Research J 4: 587, 1939, E. N. Todhunter.
(60) The Vitamins, A.M.A Public , pp 372–374. O. A. Bessey.
(61) J. Nutrition 23 239, 1942, H. A Waisman, L. M. Henderson, J. M. McIntire, and C. A. Elvehjem (Panto)
(62) Food Research J 2 549, 1937; K M Curran, D. K. Tressler, C. G. King.
(63) Am. J. Pub Health 26 905; D K Tressler, G. L. Mack, C. G. King.
(64) J. Nutrition 14 631, 1937, F. Fenton, D. K. Tressler, S. C. Camp, C. G. King.
(65) J. Nutrition 26 477, 1943; V H Cheldelin, A. M Woods, R. J. Williams.
(66) J.A.M.A 123· 902, 1943; George Kitzes, C. A. Elvehjem.
(67) J. Nutrition 21 589, 1941; L. M. Henderson, H. A. Waisman, C. A. Elvehjem (B_6).
(68) J. Nutrition 23: 417, 1942, L. J Teply, F. M. Strong, C. A. Elvehjem (Niacin).
(69) J. Nutrition 25: 275; 1943; W C. Russell, M. W. Taylor, J. F. Benk (Niacin).
(70) M.I.T. Rest. results Report Comm. N.R.C.; R. S. Harris.
(71) Science 98· 188, 1943 (Aug. 27); P. R. Burkholder (Soy Beans).
(72) Conn. Agric. Exp. Sta. Report, Bull. 415, August 1938.
(73) Ansbacher, Vitamins and Hormones; Vol. II.

TABLE I

FOODS	VITAMIN A	VITAMIN B_1	VITAMIN B_2	NIACIN	VITAMIN C
	Int. Units (U S. P. Units)/100 gm.	*mgm /100 gm.*	*mgm./100 gm.*	*mgm./100 gm.*	*mgm /100 gm.*
Almonds	75 (13)	0 225–0 250 (1) (13)	0.500 (13)	1.820 (4)	
Apples	50–147 (1) (13) (15) (39)	0 025–0 096 (1) (2) (12) (13)	0 016–0 072 (1) (2) (3) (13) (14) (65)	0 050–0 091 (2) (3) (68) (69)	4–5 (1) (13) (16) (47) (60)
Apple Juice					2 (47)
Apricots					
fresh	4000 (1) (13)	0.030–0 060 (1) (13)	0.043–0.090 (1) (13)	0.136 (69)	1–6 (1) (13) (60)
dried	5000–5800 (1) (13)	0 090–0.170 (1) (13)	0 090–0.170 (1) (13)		3–8 (13) (60)
Artichoke					
Globe	200 (13)	0 180–0 280 (1) (13)			9 (13)
Jerusalem		0.150–0 230 (1) (13)			6 (13)
Asparagus					
green	370–1400 (13) (26) (39)	0 177–0 210 (1) (12) (13)	0.100–0.129 (13) (14)	1.100–1.200 (69)	35–60 (1) (13) (39) (60)
white	0–50 (13)	0.150 (13)			31 (13)
Avocados	100–130 (1) (13)	0 090–0 102 (1) (12) (13)	0.075 (13)	0 927–1.020 (69)	20–30 (1) (13)
Bacon		0 370–0 480 (1)	0.130–0 314 (2) (3) (65)	1.300–4 200 (2) (3) (65)	
Bamboo shoots				0.200 (4)	
Bananas	147–370 (13) (18) (39)	0 042–0.160 (2) (12) (13) (18)	0.056–0.084 (2) (3) (13) (14)	0.580–0.610 (2) (3) (4) (68)	8–11 (13) (17) (60)
Barley	0 (13)	0.360–0 510 (1) (13)	0 007 (13)	4 700 (4) (68)	0 (13)
Beans					
snap	366–1400 (1) (13) (24) (39)	0 063–0 094 (1) (3) (12) (13) (28) (70)	0 090–0.161 (1) (5) (13) (14) (28)	0.350–0.640 (4) (68) (69)	2–45 (1) (13) (22) (28) (39) (60) (61) (70)
wax	800–1240 (1) (39)	0 075–0 090 (1) (12) (13)	0 080–0.101 (1) (13) (14)	0.600–0 760 (4) (68)	15–20 (1) (13)
lima	400–2000 (13) (26) (39)	0.270–0 345 (1) (12) (13)	0.130–0 250 (3) (13)	0 290–0.980 (3) (4) (65)	30–41 (1) (13) (21)
soy	200–1175 (1) (13) (33)	0 470–0 640 (1) (12) (13) (71)	0 250–0.350 (13) (71)	4 000 (71)	21–40 (1) (13) (71)
broad				2 100 (4)	
dried kidney	0 (1)	0.450–0 520 (1) (13)		2.100 (4)	
dried lima	100 (13)	0.510–0 530 (2) (12) (13)	0 140–0.750 [illegible] (13)	0 980–1.830 (2) (4)	0 (13)
dried mung				2 300 (4)	
dried navy	0 (13)	0 384–0 510 (12) (13)	0 300–0 324 (5)		0 (13)
dried soy	100–130 (1) (13)	0.900–2.000 (1) (12) (13) (71)	0 230–0.750 (13) (71)	2 000–4.320 (4) (71)	

Beef					
lean	0-50 (1) (13)	0 063-0 200 (1) [illegible] [illegible] (13)	0.159-0 [illegible] (1) (2) (3) (5) (13) (65)	4 500-6 390 (2) (3) (4) (65)	
round					
Beer		0 009 (1)			
Beets	0-100 (1) (13) (39)	0 025-0 050 (1) (2) (3) (12) (13)	0 023-0 055 (1) (2) (3) (5) (13) (14)	0 522-0 650 (2) (3) (4) (65) (69) (69)	5-15 (1) (13) (60)
greens		0 140 (2)	0 093-0 375 (2) (3) (13) (14) (65)	0 290-0 [illegible] (2) (3) (65) (69)	30-50 (1) (13) (60)
Blackberries	80-580 (1) (13) (39)	0 022-0 045 (1) (3) (12) (13)			3-7 (1) (13) (60)
Blueberries	[illegible] (1) (13) (19) (39)	0 045 (1) (12) (13)			4-15 (1) (47) (60)
Brains					
beef		0 180-0 230 (1) (2)	0.140-0 750 (1) (2)	3 200-4 900 (2) (4)	
pig				6 400 (4)	
sheep				6 000 (4)	
Bran, wheat				3 370-4 000 (68)	
Brazil nuts	10 (13)	1 000-1 050 (1) (13)			
Bread					
rye	trace (13)	0 160-0.210 (1) (3) (13)	0 072 (3)	0 920 (3)	
ord. white	trace (13)	0 047-0 060 (3) (8) (13)	0 050-0 090 (3) (13)	0.600-0.950 (3) (4) (8) (68)	
enriched white	trace (13)	0 240-0 450 (8)	0 130-0 260 (8)	0 600-4 420 (2) (8) (68)	
whole wheat	trace (13)	0.230-0 300 (2) (8) (13)	0 067-0 120 (2) (3) (13) (8)	0 910-2 800 (2) (3) (4) (8)	
Broccoli	1300-9000 (13) (39)	0.100 (1) (12)	0.210-0.226 (1) (14)	0 835-1 050 (4) (69)	13-50 (1) (13) (20) (60)
Brussels sprouts	200-290 (13) (39)	0 170-0 180 (1) (12) (13)			50-140 (1) (13) (60)
Buckwheat		0.450 (13)		4 400 (4) (69)	
Burdock greens					
Butter	2400-4600 (1) (13)	0.003 (1)	0 012 (2)		[illegible] (1)
Cabbage	20-120 (1) (13) (39)	0.037-0 168 (1) (2) (3) (13) (70)	0 038-0 075 (1) (2) (3) (5) (13) (14) (65)	0 210-0 290 (2) (3) (4) (65) (68) (69)	31-100 (1) (3) (13) (29) (60) (68)
Chinese	2000 (13)	0 075 (12)			40 (13)
Savoy		0.210 (1)			35 (1)
Canteloupe	300 (13)	0 048-0.180 (2) (3) (13)	0 026-0 073 (2) (3) (13) (14)	1 000-1.010 (2) (3)	30-40 (13) (20) (60)
Carrots					
fresh	1700-14400 (1) (13) (39)	0 049-0.130 (1) (2) (3) (12) (13) (70)	0.050-0 070 (1) (2) (5) (13) (14)	0.200-1.470 (2) (3) (4) (65) (69)	1-7 (1) (13) (60) (70)
dried					
Cauliflower	30-70 (1) (13) (39)	0.092-0.190 (1) [illegible] [illegible] (13) (70)	0 061-0 800 (1) (2) (3) (13) (14) (65)	0 530-0 570 (2) (3) (65) (69)	75-93 (1) (13) (20)

TABLE I—*Continued*

FOODS	VITAMIN A	VITAMIN B1	VITAMIN B2	NIACIN	VITAMIN C
	Int. Units (U. S. P. Units)/100 gm.	*mgm./100 gm.*	*mgm./100 gm.*	*mgm./100 gm.*	*mgm./100 gm.*
Celery	0–23 (1) (13) (39)	0 030–0 040 (1) (12) (13)	0 035 (1) (14)	0.182–0.284 (69)	5–8 (1) (13) (60)
Chard	9000 (13)		0 075–0.140 (1) (13) (14)		33–43 (1) (3) (64)
Cheese					
Brick			0 420 (7)	0.110 (7)	
Cammembert	3600 (1)		0.830 (7)	1 600 (7)	
Chantille			0.470 (7)	0.050 (7)	
Cheddar	1700–2000 (1) (13)	0 026–0.045 (1) (2) (3) (12) (13)	0.400–1.375 (2) (3) (5) (7) (13) (52)	0.020–0.200 (2) (3) (4) (5)	
Colby			0.510 (7)	0 070 (7)	
Cottage	500 (13)	0 020 (1)			
Cream	2100–2200 (1) (13)		0 140–0 200 (7)	0.060 (7)	
Edam	1800 (1)				
Old English			0 570 (7)	0.030 (7)	
Gorgonzola		0 010 (1)			
Gruyere		0.008 (1)			
Leiderkrans	3400 (1)				
Limburger	1400 (1)		0 350 (7)	0.140 (7)	
Parmesan		0 000 (1)	0.710 (7)	0.110 (7)	
Pimento	2400 (1)				
Roquefort	4000 (1)	0 030 (1)	0.450 (7)	1.240 (7)	
Stilton		0 025 (1)			
Swiss	2300 (1)		0 400–0.800 (7) (52)	0 070 (7)	
Valenta			0 [illegible] (7)	0.140 (7)	
Cherries	200–1600 (13) (39)	0 045–0.050 (1) (13)		0.127–0.145 (69)	
black					6 (1)
Chestnuts		0 270 (1)	—	1.170 (4)	
Chicken		0 080 (3)	0 276–0.342 (37)	4.200–13 500 (4)	
white meat		0.074–0.090 (1) (2) (12) (13)	0 120–0 130 (2) (3) (65)	7.300–9.400 (2) (3) (4) (65)	
dark meat		0.090–0 150 (1) (2) (12) (13)	0 247–0 342 (2) (3) (37) (65)	3.190–8.140 (2) (3) (4) (65)	
Chives					50 (1)
Chocolate		0.047–0.640 (2) (3)	0.240 [illegible] (3)	1.100 (2) (3)	
Cider					
Clams	14 (3)	0 021 (13)	0.120 (1)	0 108 (68)	5 (1)
Cocoa		0 070–0 075 (12)			

Coconut		0 030 (1)		0 400 (4)	1 (1)
Cod	5 (13)	0 090-0.120 (1) (13)	0 050 (1)	2 300 (4)	2 (1)
Collards	7000-8800 (1) (13)	0 150-0 200 (1) (12) (13)	0 250 (13)		40-60 (13) (60)
Corn					
sweet	230-2300 (1) (13) (39)	0 120-0 160 (1) (12) (13)	0 050-0 121 (13) (14)	1 300 (3)	7-14 (1) (13) (34) (39) (50)
germ					
Cornmeal					
white		0 300-0 [illegible] (1) (12) (13)	0 063 (3)	0 176-0 900 (3) (4) (63)	
yellow	[illegible] (13)	0 230-0.300 (1) (12) (13)	0 035 (3)	0 103-0 [illegible] (4) (63)	
Cottonseed meal					
Cowpeas					
fresh	310-600 (1) (33)				
dried	30-50 (1) (33)	0 810-0 936 (1) (3) (12) (13)	0 140-0 250 (2) (13)	1.300 (3)	
Crabs		0 230 (1)	0 150 (1)	2.800 (4)	
Crackers, graham		0 070 (1)			
Cranberries	20-70 (1) (13) (39)		0 000 (13)	1 290 (4)	0-12 (13) (60)
Cream 20%	600 (13)	0 [illegible] (13)			
Cream					
20%	600 (13)	0 030 (13)			
40%	1200 (13)				
Cucumbers	0-20 (13)	0 045-0 050 (1) (13)	0.020-0 045 (1) (13) (14)	0.109-0 [illegible] (4) (63) (69)	2-10 (13) (60)
Currants					
red	120-400 (1) (13)	0 030-0 045 (13)			15-45 (1) (13) (60)
black					100-180 (1) (60)
Dandelion greens	12000 (13)	0.160 (1)			40-100 (13) (60)
Dates					
fresh	60-150 (1) (13)	0.070-0 075 (1) (12) (13)		2.180 (4) (63)	0 (13)
dried	150	0.075 (13)			0 (60)
Egg Plant	0-35 (1) (13)	0 045-0 053 (3) (13)	0.025-0 [illegible] (1) (13) (14)	0.600 (4)	5-12 (1) (13) (47) (60)
Eggs					
whole hen's	1000 (1) (13)	0.112-0 260 (1) (2) [illegible] (13)	0.190-0 520 (1) (2) (3) (5) (14)	0 072-0 [illegible] (2) (3)	0 (1) (13) (60)
white	0 (13)	trace (12)	0 250-0.400 (1) (13)	0.078 (4) (63)	0 (1) (13)
yolk	2600-2800 (1) (13)	0 220-0 420 (1) (3) (12) (13)	0 288-0 760 (1) (13)	0 035 (4) (63)	0 (13)
Endive	3500-27000 (1) (13) (39)	0 060-0 099 (1) (12) (13)	0 060-0 214 (1) (5) (13) (14)	0.720 (4)	10-15 (1) (13) (20) (60)
Escarole					7 (60)

TABLE I—*Continued*

FOODS	VITAMIN A	VITAMIN B_1	VITAMIN B_2	NIACIN	VITAMIN C
	Int. Units (U. S. P. Units)/100 gm.	*mgm./100 gm.*	*mgm./100 gm.*	*mgm./100 gm.*	*mgm./100 gm.*
Farina				0.980 (68)	
Figs					
fresh	150 (13)	0.060–0.075 (1) (13)	0.038 (13)	0.[illegible] (4)	2 (1) (13) (60)
dried	[illegible] (13)	0.066 (13)	0.138 (13)	1.720 (4)	0 (13) (60)
Filberts		0.818			
Flounder	trace (13)	0.084 (3)		3.840 (4)	
Flour					
rye	0 (13)			0.730–1.220 (4)	
patent wheat	0 (13)	0.050–0.090 (1) (12) (13)		0.800 (4)	
enriched	trace (13)	0.073 (2)	0.036 (2)	0.610 (2)	
wholewheat		0.450–0.690 (1) (13)		0.500–0.700 (68)	
Grapefruit	0 (13) (39)	0.059–0.150 (3) (12) (13)	0.030 (2) (3)	0.154–0.210 (2) (3) (69)	35–42 (1) (13) (36)
juice	0 (13)	0.032–0.075 (11) (13)	0.011 (11)	0.154–0.210 (68) (69)	40–46 (13) (40) (60)
Grapes					
Concord	15–90 (1) (13) (39)	0.045–0.067 (1) (3) (13)	0.020–0.092 (1) (13) (14)	0.840 (4)	3–4 (1) (13)
California				0.280 (68)	
Grape juice		0.030 (1)	0.022–0.100 (1) (41)		2–6 (1) (13)
Grass				0.780 (68) dried	[illegible] (60)
Guavas	200 (13)	0.042 (13)	0.008 (13)	1.100 (4)	75 (13)
Garlic					15 (1)
Gluten wheat				0.250 (68)	
Gooseberries	380 (1)				25–35 (1) (13) (60)
Haddock	0–5 (1) (13)	0.009–0.015 (1) (13)			
Halibut		0.073–0.092 (1) (2) (3) (12) (13)	0.044–0.088 (2) (3) (5)	6.030–11.000 (3) (4) (65)	
Hazelnuts		0.230–0.6[illegible] (1) (13)			
Heart					
beef		0.440–0.670 (12) (2) (13)	0.750–0.890 (1) (2) (3) (13) (65)	7.600–8.700 (2) (3) (4) (65)	
chicken				7.400 (4)	
pig		0.540–0.590 (1) (13)		7.300 (4)	8 (1)
sheep	trace (13)	0.600 (13)		3.400 (4)	4 (1)
calf				1.060 (4)	
Herring				3.500 (4)	

Hominy					
grits			0 055 (2)	0 200 (2)	
Honey	0 (1)	0 0036-0 0031 (9)	0 062-0 [illegible] (9)	0 200-0 400 (9)	2 (9)
Horseradish					100-120 (1) (13) (60)
Kale	10000-45000 (1) (13) (39)	0 150-0 190 (1) (12) (13)	0 500 (13)		50-147 (1) (13) (20) (60)
Kidney					
beef	1000 (13)	0 180-0 320 (1) (13)	1 730-2 109 (1) (13)	0.734 (4)	
chicken					
lamb					
pig		0 450-0 520 (1) (13)	2 000 (1)	9 900 (4)	
sheep	1000 (13)	0 225-0 230 (1) (13)		6 000 (4)	
calf	1000 (13)	0 150 (13)	1 730 (13)		
Kohlrabi		0 960 (13)		0 270 (1) (69)	60-70 (13) (20) (60)
Kumquats					20 (1)
Lamb					
lean		0 240-0 440 (1) (13)	0 175-0 330 (1) (13) (65)		
leg		0 280 (2)	0.240 (2)	7 500-8 490 (2) (3) (4) (65)	
chop		0 237-0 258 (12)			
loin		0 250 (1)			
Lard					
Leeks		0 150 (13)			15-20 (1) (13) (60)
Lemon	0 (1)	0 020 (1)	0.004 (1)	0.154-0.190 (69)	30 (1)
juice	0 (1)	0.030 (13)	trace (13)	0.030 (68)	45-60 (13) (42) (60)
Lentils, dried		0.470-0.510 (1) (13)	0.203 (13)	3.100 (4)	0 (13)
Lettuce					
head	100-700 (13) (30)	0 039-0 087 (1) (2) (3) (12) (13)	0 027-0 050 (1) (2) (13) (14)	0.100-0 300 (2) (3) (4) (69)	5-15 (1) (13) (20) (60)
iceberg			0 065 (5)		[illegible] (20) (60)
romaine	800 (13)		0 075-0 100 (1)		15-19 (1) (20)
Limes				0.227-0.275 (69)	(25) 1
juice				0.090 (68)	30-33 (13) (60)
Limequats					[illegible] (1)
Liver					
beef	59500 (1)	0 225-0 330 (1) (2) (13) [illegible] (12)	1 500-3 600 (1) (2) (3) (13) (65)	11 750-120 000 (2) (30)(4) (65)	10-30 (1)
calf	7000-106000 (1) (13)	0 150-0 210 (1) (13)	1.375-3 600 (1)	17 [illegible] (4)	
chicken		0.225 (13)	2 650 (37)	8.000 (4)	[illegible] (1)
goose					
lamb	90300 (1)	0 225-0 410 (1) (13)	1 375-4 300 (1) (13)	17 600 (4)	

TABLE I—*Continued*

FOODS	VITAMIN A	VITAMIN B_1	VITAMIN B_2	NIACIN	VITAMIN [illegible]
	Int. Units (U. S. P. Units)/100 gm.	*mgm /100 gm.*	*mgm./100 gm.*	*mgm./100 gm.*	*mgm./100 gm.*
Liver—Cont'd					
pig	27800 (1)	0.300-0.1500 (1) (13)	1.500-2.700 (1) (13)	22.800 (4)	
sheep			4.300 (1)		
turkey	60500 (1)				
Lung					
beef				6.200 (4)	
pig				2.500 (4)	15 (1)
sheep				3.700 (4)	45 (1)
Loganberries					35 (1)
Macaroni				2.100 (68)	
Mackerel	170 (1)			5 500 (4)	
Malt sprouts				0 560 (68)	
Mangels				0.130-0 [illegible] (4)	
Mangoes	1500 (13)	0.090 (13)	0.050 (13)		30 (13)
Melon, honeydew		0.046 (3)			
Milk					
whole	110-200 (1) (13)	0 030-0.060 (1) (2) (12) (13)	0.095-0.270 (1) (2) (3) (5) (13)	0 066-0.1[illegible] (2) (3) (4) (68)	[illegible] (1) (6)
skim	2-15 (1) (13)	0 040-0.045 (1) (12) (13)		0 060-0.090 (4)	0 (13)
whole dried	875-1600 (1) (13)	0.276-0 360 (1) (3) (12) (13)	1.260-2.600 (1) (13)	0 880-0.890 (4) (68)	0-8 (1) (13)
skim dried	20-140 (1) (13)	0 260-0.375 (1) (12) (13)			0-7 (1) (13)
butter					
evaporated	460 (1)	0.040-0.051 (1) (12)		0.180 (4) (68)	1 (1)
human				0 [illegible] (68)	6 (60)
acidophilus				0.080 (68)	
Millet		0 720 (1)		0 800 (4)	
Molasses	0 (1)	0 050-0.590 (1) (2)	0 062-0.160 (1) (2) (3)	3.900 (2) (3)	
Mushrooms	0 (1)	0 090-0.110 (1) (13)	0 330 (2)	6.900 (2)	8 (1)
Muskmelons	2400 (1)	0.057-0 060 (1) (12)	0.070 (1)		35 (1)
Mustard greens	10200 (1)	0.135-0.140 (1) (12)			60-170 (1) (13) (60)
Mutton		0.051-0.100 (1) (2) (3)	0.248-0 250 (2) (3) (65)	4 000 (2) (3)	
shoulder					
breast					

Oats	trace (13)	0 440–0 726 (1) [illegible] [illegible]	0 059–0.100 (5) (13)	1 100–1 [illegible] (4) (63)	
rolled	0 (1)		0 059–0.100 (5) (13)	0 [illegible] (4)	
Oatmeal	0 (1)				
Oils					
Corn	2 (13)				
Cottonseed	0 (13)				
Cod Liver	85000 (1)				
Fish					
Halibut Liver					
Shark					
Tuna fish					
Soy bean					
Olive	0 (13)				
Okra	400 (13)			0 693–0.710 (3) (63)	10–20 (1) (3) (60)
Oleomargarine					
fortified	197 (1)				
Olives					
green	190–1000 (1) (13)				0 (13)
ripe	125 (13)	0 008 (13)			0 (13)
Onions	0 (1) (13) (39)	0 023–0 030 (1) (13) (12) (13) (65)	0.024–0 123 (1) (3) (13) (14)	0 100–0 132 (4) (65) (63)	8–27 (1) (13) (44) (60)
dried		0 036	0 190 (2)	0 090 (2)	
Oranges	65–250 (1) (39)	0 067–0.100 (1) (2) (3) (12)	0 030–0 047 (1) (2) (3)	0 173–0 300 (2) (3) (69)	45–49 (1) (38)
juice	45–350 (13)	0 065–0 150 (11) (13)	0 013–0 016 (3) (11)	0 145–0 220 (69) (69)	23–60 (13)
Oysters	140–210 (1) (13)	0.180 (2)	0.220 (2)	1.200–1 300 (2) (4)	
Pancreas					
beef		0 320 (1)	0 500 (1)	0 534 (4)	15 (1)
pig				0 500 (4)	
sheep				4 000 (4)	
Papayas	2500 (1) (13)	0 025–0.075 (13)	0 150 (13)	0.091 (69)	40–70 (1) (13) (60)
Paprika				1 330 (69)	
Parsley	30000 (13)				100–193 (1) (13) (30) (60)
Parsnips	trace (13)	0.110–0.120 (1) (13)			5–40 (1) (13) (20) (46) (60)
Peaches					
white	5 (1)	0 005–0 030 (1) [illegible] [illegible] (13)	0.050–0 065 (1) (2) (13) (14)	0.330–0.950 (2) (3) (4) (68)	7–8 (1) (60)
yellow	1000–3000 (1) (13) (39)				
dried	2400–3000 (1) (13)				

TABLE I—*Continued*

FOODS	VITAMIN A	VITAMIN B1	VITAMIN B2	NIACIN	VITAMIN C
	Int. Units (U. S. P. Units)/100 gm.	*mgm./100 gm.*	*mgm./100 gm.*	*mgm./100 gm.*	*mgm./100 gm.*
Peanuts					
raw		0.960–1 100 (1) (12) (13)	0 110–0 625 (3) (13)	5.900–8 [illegible] (3) (4)	
roasted		0.234–0 370 (1) (12) (13)	1 050 (2)	8.600 (2)	
red skins		7 [illegible] (12)			
butter		0.230 (1)		18 [illegible] (4) (68)	
meal					
germ		0 882 (12)			
Pears	10–50 (1) (13) (39)	0 025–0 045 (1) (12) (13)	0 050–0 076 (1) (13) (14)	0 091–0 140 (4) (66) (69)	3–9 (1) (13) (45) (60)
dried					
Peas					
green	600–3300 (13) (39) (58) (59)	0.350–0 420 (1) (2) (13) (70)	0.134–0 200 (1) (2) (5) (13) (14)	0.700–1.520 (2) (4) (69)	18–28 (1) (13) (39) (55 (60) (63)
dried	1200 (13)	0.525–0.610 (2) (3) (13)	0 160–0 250 (2) (13) (3) (65)	1.800–2.800 (2) (3) (4)	
chick				3.690 (4)	
Pecans	200–400 (13)	1.050 (13)	0 250 (13)		
Peppers					
green	800–700 (1) (13) (39)	0 030–0 060 (1) (13)	0 030–0.138 (5) (14)	0.220 (4)	180–200 (1) (60)
red					230 (60)
Persimmons	2500 (1)				45 (1)
Pineapples	90–200 (1) (13) (39)	0.075–0.090 (12) (13)	0 030 (13)		25–45 (1) (13) (60)
juice		0.150 (13)		0 [illegible] (69)	[illegible] (13)
Plums	[illegible] (1)	0.048–0 105 (1) (12) (13)	0 [illegible] (13)	0.560 (4) (68)	2–6 (1) (13) (47) (60)
Pokeweed					
Pomegranates					6 (1)
Pork					
lean raw			0.188–0 [illegible] (3) (5) (13)		
chop		0.188–0 [illegible] (3) (5) (13)	0 270 (3)	6 000 (3)	
loin		0 770–1 700 (1) (2)	0 147–0 [illegible] (2) (5) (65)	3.130–9.114 (2) (4) (65)	
shoulder		1 051 (3)			
ham		0 920–2.300 (1) (2) [illegible]	0 200–0 270 (2) (3) (5) (65)		
ham smoked		1 300–1.428 (1) (12)	0 230–0 290 (5)	8.200 (4)	
ham cured					
headcheese		0 058 (3)			
sausage		0 260 (1)			

Potatoes					
white	30-50 (1) (13) (39)	0 054-0 188 (1) (3) (12) (13) (70)	0 025-0 065 (1) (2) (3) (5) (13) (14)	0 365-1 430 (2) (3) (65)	7-15 (1) (13) (60)
dried white					6 (1)
sweet	1000-7000 (1) (13) (39)				8-25 (1) (13) (60)
Prunes					
fresh			0 025 (3)		
dried	1400-3600 (1) (13) (39)	0 150-0 190 (12) (13)		0 330-0 453 (62)	3 (13) (60)
Pumpkin	2500-20000 (13) (39)	0 045 (13)	0 [illegible] (13)	0 700 (4)	3-5 (13) (60)
Quinces					
Radishes		0 020-0 060 (1) (3) (13)	0 025-0 035 (13) (14)	0 100-0 164 (4) (69)	12-25 (1) (13) (60) (63)
Raisins	0-50 (1) (13)	0 090-0 230 (1) (2) (3) (13)	0 [illegible] (2) (3)	0 270-0 630 (2) (3) (4) (63)	
Rape					0 (13) (60)
Raspberries	130-520 (1) (39)	0 025-0 030 (1) (13)			15-[illegible] (1) (13) (47) (60)
Red snapper					15-20 (1) (13) (60)
Rhubarb	100 (1)	0 010 (1)			
Rice					
brown	0 (1)	0 225-0 420 (1) (13)	0 075-0 120 (1) (13)	0 600 (4)	
polished	0 (1) (13)	0 020-0 030 (1) (13)	0 053 (65)	0 650-0 900 (4) (65)	
polish				30 000	
Roe					
cod	2000 (13)	0 090 (13)			5-45 (1) (13)
shad					
Romaine	800 (13)		0.098 (14)		
Rutabagas	0-25 (13) (39)	0 045-0 075 (1) (12) (13)			20-30 (13) (60)
Rye	0 (1) (13)	0 420-0 470 (1) (12) (13)		1,220-1 [illegible] (4) (68)	
Sage					25 (1)
Salmon	260 (1)	0 110-0.130 (1) (2)	0.140-0.144 (2) (3) (65)	6 000-6.400 (2) (3) (4) (65)	
Sardines	280 (1)	0 030 (13)	0 480 (1)	2 000-3 500 (4)	
Sauerkraut		0 030 (1) (12)	0 040 (65)	0 170 (65)	30 (1)
juice					5-8 (1) (60)
Sausage					
bologna					
liver					
Scallops	0 (1)				
Shallots					

TABLE I—*Continued*

FOODS	VITAMIN A	VITAMIN B1	VITAMIN B2	NIACIN	VITAMIN C
	Int. Units (U. S. P. Units)/100 gm.	*mgm./100 gm.*	*mgm./100 gm.*	*mgm./100 gm.*	*mgm./100 gm.*
Shepherd's purse					20 (1)
Shrimp			0.150 (1)	0.780 (4) (68)	
Sole		0.090 (1)			
Spaghetti				2.100 (4)	
Spinach	6600–75000 (1) (13) (39)	0.056–0.120 (1) (2) (3) (12) (13)	0.160–0.312 (1) (2) (3) (13) (14)	0.510–0.782 (2) (3) (4) (62) (65) (69)	28–99 (1) (13) (20) (39) (60) (63)
Squash					
summer	1000 (13)	0.040–0.069 (1) (12) (13) (14) (70)	0.038 (13)		
winter	*4000–20000 (13) (39)*	*0.045–0.[illegible] (1) (12) (13) (70)*	0.045–0.063 (1) (13) (14)		1–5 (1) (13) (60)
succini				0.960 (4)	
Strawberries	50–740 (1) (39)	0.021–0.035 (1) (2) (3)	0.180–0.340 (1) (2) (3)	0.220–0.264 (2) (3) (69)	36–70 (1) (13) (47) (50) (56) (60)
juice					55–60 (47)
Suet, beef	*600 (1)*				
Sweetbreads		0.040 (1)			
Sweet potatoes	1000–7000 (1) (13) (39)	0.090–0.120 (1) (2) (3) (12) (13)	0.040–0.075 (1) (2) (5) (13) (14) (65)	0.450–0.790 (2) (65) (69)	8–25 (1) (13) (60)
Tangerines	[illegible] (1)	0.[illegible] (13)	0.025 (13)		30–35 (1) (13) (60)
juice		*0.069 (11)*			
Tapioca			0.420 (1)	0.300 (4)	
Tomatoes	500–3000 (1) (13) (39)	0.060–0.[illegible] (1) (2) (12) (13)	0.037–0.052 (1) (2) (13) (14)	0.230–0.580 (2) (68) (69)	22–30 (1) (13) (60) (63)
juice	1000 (13)	0.075 (13)		0.100–0.564 (68) (69)	20–30 (1) (13) (51) (60)
Tongue					
beef		0.280 (1)		7.100 (4)	
pig				5.300 (4)	
Tripe		0.006 (1)			
Trout		0.087–0.090 (1) (12)			
Tuna	[illegible] (1)				
Turbot		0.020 (1)	0.140 (1)		
Turnips	0–40 (13) (39)	0.026–0.128 (2) (3) (12) (13) (70)	0.030–0.065 (1) (2) (3) (13) (14) (65)	0.600–0.990 (2) (3) (65)	30–42 (1) (13) (47) (60)
greens	10000–15700 (1) (13)	0.120–0.140 (1) (12) [illegible]	0.300 (13)		60–160 (1) (13) (60)

Veal					
chop		0 083 (2)	0 140-0 220 (2) (3) (65)	7.100-7 200 (2) (3) (65)	
quarter		0 290 (1)	0 240 (2)	8 400 (4)	
chuck		0 073 (3)			
Vegetable juices:					
cabbage	3800 (54)				33 (54)
carrot	7800 (54)				4 (54)
celery	708 (54)				5 (54)
green bean	730 (54)				
spinach	9000 (54)				27 (54)
Walnuts	*40-130 (13) (1)*	0 330-0 342 (1) (12) (13)			
English	100 (13)	0 450 (13)			
Water					
cress	4000 (13)	0 130 (13)	0 225 (13)		50-75 (1) (13) (60)
Watermelon	[illegible] (1)	0 027-0 070 (1) (2) (3) (13)	0 025-0 069 (2) (3) (13)	0 240 (2) (3)	6-15 (1) (13) (60)
Wheat					
whole grain	trace (13)	0 354-0 540 (2) (12) (13)	0 089-0 180 (2) (5) (13)	4 000-4 600 (2) (4)	
bran . .		1 400 (1)		4.200 (4)	
germ . .		1 800-2 600 (1) (2)	0 490 (2)	2 200-4 [illegible] (2) (4)	
Whey, dried			2 700 (1)		
Wines					
Yams				0 670 (68)	
Yeast,					
bakers' moist .		0 910 (1)	1.800 (1)	11.000-60 000 (4) (69)	
dried		17700 (1)	6.200 (1)	40.000 (4)	
brewers' fresh					
dried . .		16300 (1)	3.700 (1)	40.000 (4)	
primary					
grown dried.					
cakes.					
Youngberries	460 (1) (39)			40.000-80 000 (83)	
juice .					

TABLE II

The daily requirement of three of the B Complex factors (Thiamine or B_1, Riboflavin or B_2, and Niacin) is now fairly well established. See Table VIII and IX Chapter IV for amounts and Table I of the Appendix for distribution in common foods.

For other member of the B Complex, human needs have not yet been established and assays are limited. The following tabulation gives amounts reported in types of foods and gives some idea of relative richness of food types in the factors listed.

FOODS	MILLIGRAMS PER 100/GM. RAW FOODS OF						
	Pantothen	Pyridoxine	Inositol	Biotin	Folic acid	Choline	Paba
Meats							
Bacon	0 280-1 000 (2) (3) (65)	0 029 (2)	43-64 (2) (65)	0 0074-0.008 (2) (65)	0 006-0.139 (2) (65)	?	?
Beef (lean)	0 490-1 300 (2) (3) (61)	0 077-0.350 (2) (67)	4.5-11.5 (2) (65)	0.0026 (2) (65)	0.100-0.105 (2) (65)	95 (6)	?
Brains (beef)	1 800-3 600 (2) (61)	?	200 (2)	0.0074 (2)	0 052 (2)	?	?
Chicken,							
dark	0 [illegible] (2) (3) (65)	0 025 (2)	37-47 (2) (65)	0 0093 (2)	0.120-0.149 (2) (65)	?	?
light	0 530-0 900 (2) (3) (61)	0.130 (2)	48 (2)	0.0054 (2)	0.150-0.203 (2) (65)	?	?
Heart, beef	2 000 (2) (3) (61) (65)	0 120-0 240 (2) (67)	260 (2)	0 0049-0.008 (2) (65)	0 110-0.160 (2) (65)	?	?
Lamb	0 600-1 300 (3) (61)	0.300 (67)	58 (65)	0.002 (65)	0 115 (65)	107-119 (6)	?
Liver, beef	*4 400-7.800 (2) (3) (61) (65)*	*0.170-0 730 (2) (67)*	*55 (65)*	*0 096-0.112 (2) (65)*	*0 825-0 350 (2) (65)*	*10-30 (6)*	?
Mutton	0.430 (2) (3)	0.081 (2)	50-51 (2) (65)	0.0027 (21) (65)	0.077 (2) (65)		
Pork							
loin	1 500-2.100 (2) (61)	0 086 (2)	200 (2)	0 0020-0 0046 (2)	0 053-0.084 (2) (65)		8 ppm (72)
ham	0 340-0 500 (2) (3) (65)	0 019-0 590 (2) (67)	[illegible] (2) (65)	0.0060 (2) (65)	0.058 (2) (65)		
Veal chop	0 110-0.280 (2) (3) (65)	0.056 (2)	32-36 (2) (65)	0 0014-0 0064 (2) (65)	0 092-0.160 (2) (65)	113 (6)	
Fish							
Halibut	0.150 (2) (3)	0 110 (2)	17-19 (2) (65)	0 0080-0.0120 (2) (65)	0 054-0 071 (2) (65)	?	?
Oysters	0 490 (2)	0 033 (2)	44 (2)	0 0087 (2)	0.240 (2)	?	?
Salmon	0 [illegible] (2) (3) (65)	0.033 (2)	[illegible] (2) (65)	0.0053-0 0254 (2) (65)	0 [illegible] (2) (65)	?	?
Dairy Products							
Egg	1.400 (2) (3)	0.022 (2)	33 (2)	0 0090 (2)	0.086 (2)	?	?
Milk	0.290 (2) (3)	0.130 (67)	18 (2)	0 0050 (2)	0 005 (2)	14.7 (6)	1 ppm (72)
Cheese, Cheddar	0.130-0.230 (2) (3) (7)	0 066 (2)	25 [illegible]	0.0036 (2)	0 [illegible] (2)	48 (6)	?
Cereal Products							
Bread							
white	0 460 (2)	0 0039 (2)	51 (2)	0.0011 (2)	0 034 (2)	?	?
enriched							
wh. wheat	0 420-0 570 (2) (3) (8)	0 038 (2)	67 (2)	0.0019 (2)	0 069 (2)	?	?
Flour							
white	0 350 (2)	0 020 (2)	74 (2)	0 0007 (2)	0 067 (2)	?	?
enriched							

Cornmeal, white	0.310 (2)	0.054 (2)	[illegible] (2)	0.0059 (2)	0.020 (2)	?	?
Hominy grits	0.310 (2)	0.054 [illegible]	3.2 (2)	0.0005 (2)	0.010 (2)	?	?
Wheat, whole	1.200 (2)	0.210 (2)	170.2 (2)	0.0052 (2)	0.170 (2)	?	5 ppm (74)
Wheat germ	2.000 (2)	0.600 (2)	690 (2)	?	1.100 (2)	?	1.5 ppm (72)
Fruits							
Apples	0.000 (2) (3)	0.026 (2)	24 [illegible]	0.0009 (2)	0.009 (2)	?	?
Bananas	0.150 (2) (3)	0.320 (2)	34 (2)	0.0044 (2)	0.023 (2)	?	?
Canteloupe	0.230 (2) (3)	0.036 (2)	120 (2)	0.0031 (2)	0.130 (2)	?	?
Grapefruit	0.200 (2) (3)	0.009 (2)	150 (2)	0.0020 (2)	0.055 (2)	?	?
Oranges	0.340 (2) (3)	0.050 (2)	210 (2)	0.0019 (2)	0.053 (2)	?	?
Peaches	0.170 (2) (3)	0.016 (2)	96 (2)	0.0017 (2)	0.017 (2)	?	?
Raisins	0.000 (2) (3)	0.094 (2)	120 (2)	0.0031 (2)	0.025 (2)	?	?
Strawberries	0.260 (2) (3)	0.044 (2)	60 (2)	0.0040 (2)	0.023 (2)	?	?
Watermelon	0.310 (2) (3)	0.033 (2)	64 (2)	0.0038 (2)	0.150 (2)	?	?
Vegetables							
Beans							
lima	0.830 (3) (65)	?	163 (65)	0.0059 (65)	0.310 (62)	?	?
dried lima	0.830 (2)	0.550 (2)	170 (2)	0.0095 (2)	0.330 (2)	?	?
soy	1.200 (71)	0.350 (71)	?	0.0540 (71)			
dried soy	1.200 (71)	0.640 (71)	?	0.0610 (71)	?	350 (6)	?
Beets	0.110–0.130 (2) (3)	0.013 (2)	10–12 (2) (65)	0.0003–0.0011 (2)	0.045 (2) (65)	?	?
greens	0.140 (2) (3) (65)	0.037 (2)	21 (2)	0.0027 (2) (65)	0.210 (65)	?	?
Cabbage	0.150 (2) (3) (65)	0.120 (2)	95 (2) (65)	0.0024 (2) (65)	0.065 (2) (65)	235 (6)	14 ppm (72)
Carrots	0.250 (2) (3)	0.120 (2)	49 (2) (65)	0.0023 (2)	0.097 (2) (65)	?	43 ppm (72)M
Cauliflower	0.920–1.000 (2) (3) (65)	0.020 (2)	95 (2)	0.017 (2)	0.110–0.140 (2) (65)	?	?
Cowpeas, dried	1.040 (2)	0.190 (2)	330 (2)	0.018 (2)	0.740 (2)	?	?
Lettuce (head)	0.110 (2) (3)	0.071 (2)	55 (2)	0.0031 (2)	0.038 (2)	?	?
Mushrooms	1.700 (2)	0.045 (2)	17 (2)	0.016	0.[illegible] (2)	?	1.3 ppm (72)
Okra	0.210–0.247 (3) (65)	?	[illegible] (65)	0.0017 (2)	0.053 (65)	?	?
Onions	0.130 (2)	0.063 (2)	88 (2)	0.0035 (2)	0.013 (2)	?	?
Peas							
green	0.380 (2)	0.079 (2)	162 (2)	0.0094 (2)	0.130 (2)	?	?
dried	?	0.300 (2)	180–330 (2) (65)	0.0092–0.0152 (2) (65)	?	?	?
Potatoes							
white	0.320 (2) (3)	0.220 (2)	29 (2)	0.0006 (2)	0.053–0.140 (2) (65)	?	?
sweet	0.940 (2)	0.320 (2)	66 (2)	0.0043 (2)	0.[illegible] (2)	35 (6)	0.12 ppm (72)
Sauerkraut	0.054 (65)	?	3 [illegible]	0.0002 (65)	0.019 (65)	?	?
Spinach	0.180–0.180 (2) (3) (65)	0.083 (2)	[illegible] (2) (65)	0.0059–0.0069 (2) (65)	0.170–0.240 (2) (65)	233 (6)	0.6 ppm (72)
Tomatoes	0.370 (2)	0.080 (2)	46 (2)	0.0040 (2)	0.075 (2)	?	?
Turnips	0.140 (3) (65)	0.110 (2)	40–50 [illegible] (65)	0.0021 (2) (65)	0.026–0.065 (2) (65)	?	?

Vitamin A deficiency and infection

It has been known for many years that vitamin deficiency lowers resistance of the organism infection. Germ-free rats on a vitamin A-defici diet did not die before 145 days, and one lived long as 202 days, whereas the conventional r rapidly died of infection, usually after 35 to 40 d on the vitamin A-deficient diet. This work fu substantiates the generally-held belief that vitamir confers protection against infection

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